Public University of Navarra,

Department of Health Sciences

Determination of the Aerobic Capacity in Amateur to Elite Athletes and Elderly Men, with Special Reference to the Development of Functional Strategies to Overcome Actual On-Field Hitches

Doctoral Thesis

Ibai Garcia Tabar

.

June 2016

Supervisors

Esteban M Gorostiaga

Mikel Izquierdo Redín

Public University of Navarra,

Department of Health Sciences

Determination of the Aerobic Capacity in Amateur to Elite Athletes and Elderly Men, with Special Reference to the Development of Functional Strategies to Overcome Actual On-Field Hitches

Doctoral Thesis

Ibai Garcia Tabar

June 2016

Supervisors

Esteban M Gorostiaga

Mikel Izquierdo Redín

Determination of the Aerobic Capacity in Amateur to Elite Athletes and Elderly Men, with Special Reference to the Development of Functional Strategies to Overcome Actual On-Field Hitches

Ph.D. Thesis

Ibai Garcia Tabar

Department of Health Sciences

Public University of Navarre

Pamplona

upna Universidad Pública de Navarra Nafarroako Unibertsitate Publikoa

Table of Contents

Declaration

I, Ibai Garcia-Tabar, do hereby declare that the research presented in this dissertation is based on 4 articles (chapters 2 to 5) that have been published or accepted for publication in international peer-reviewed journals. To meet the stylistic requirements of a thesis, the formats of the papers have been adjusted accordingly throughout. These edits did not substantially change the content of the published articles. The role which I fulfilled within each of the publications is presented below.

Chapter 2

Garcia-Tabar I, *Eclache JP, Aramendi JF & Gorostiaga EM. Gas analyzers' drift leads to systematic error in maximal oxygen uptake and maximal respiratory exchange ratio determination. Frontiers in Physiology 2015; 30(6):308.*

EM Gorostiaga conceived the research idea and we both designed the experiments. I collected all the data. EM Gorostiaga, JF Aramendi and I Madariaga contributed to the acquisition of the data. I assembled and analyzed all the data. I prepared the figures and tables. I drafted the first version of the "Materials and Methods" and "Results" sections. EM Gorostiaga finished the first draft of the published manuscript. I critically reviewed and edited the drafts.

Chapter 3

Garcia-Tabar I, *Llodio I., Sánchez-Medina L, Ruesta M, Ibañez J & Gorostiaga EM. Heart rate-based prediction of fixed blood lactate thresholds in professional team-sport players. Journal of Strength and Conditioning Research 2015; 29(10):2794-2801.*

EM Gorostiaga conceived the research idea. The data of this project was collected during routine sport scientific support given to the participants. I participated in all testing sessions. The data was captured with assistance of JC Lizarazu, M Ruesta, L Sánchez-Medina J Ibáñez and EM Gorostiaga. I assembled and analyzed all the data. I prepared the figures and tables. I personally wrote the first draft of the published paper.

Chapter 4

Garcia-Tabar I, *Sánchez-Medina L, Aramendi JF, Ruesta M, Ibañez J & Gorostiaga EM. Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists. Journal of Exercise Physiology Online 2013; 16(5):38-50.*

EM Gorostiaga and I contributed to the conception of the research idea and designed the experiment. I collected all the data. During the experimental period, I received support from JF Aramendi. I analyzed the data and prepared the figures and tables. I personally drafted the

manuscript. Apart from the normal guidance from my supervisors, I did not receive any other assistance.

Chapter 5

***Garcia-Tabar I**, Llodio I, Sánchez-Medina L, Asiain X., Ibañez J & Gorostiaga EM. Validity of a single lactate measure to predict fixed lactate thresholds in elite athletes. Journal of Sports Sciences 2016; Epub ahead of print*

I conceived the research idea. The data of this project was collected during regular sport scientific support given to the participants during the years of 2006 to 2011. I only participated in the testing sessions of the 2010-2011 season. I personally gathered and analyzed all the data. I drafted the first version of the manuscript.

Agradecimientos

En primer lugar, quisiera aprovechar esta oportunidad para dar mis más profundos y mimosos agradecimientos a mis padres por brindarme apoyo constante y, entre otras cosas, por dar el primer paso de esta tesis al mandarme al extranjero algún que otro verano empezando cuando tan solo tenía 12 años. Lo que no se esperaban era que, a cambio, en mi verano de los 16, les pidiera 9 pantalones blancos para julio.

Gracias a mi hermana, la *Pelos*, por mantener la familia unida, y por usar junto a mí, otros 9 pantalones blancos.

Me gustaría brindar mis más sinceras gratitudes a Esteban, por atenderme y aconsejarme incluso años antes de empezar mi andadura en el CEIMD, por abrirme las puertas desde el primer día (aun sin saber a quién, o a qué, daba paso a entrar), por su constancia, optimismo y pasión; y por su admirable e ilimitada "*stamina*". Gracias a ti, ya no pongo más candados.

A todos esos compañeros del CEIMD por facilitarme mi labor todos los días sin excepción, tanto en mis días soleados como en mis días nublados. Gracias Wilkie, Maite, Jesús, Xabi, Luis, Javier, Saioa, Eder, Susana, Natalia, Cristina, Cecilia, Iñaki, Álvaro Gandalf,…

Mención distinguida para la banda del bate compuesta por Ion, Igor y Fabri por su inagotable compañerismo dentro y fuera del mundo laboral. Gracias Ion por no juzgar, y por el simple hecho de dar; gracias Igor por tu soporte, pero sobre todo por tu empuje descomunal y sostenido; gracias Fabri por tu saber estar, por mantener la atención, y aparecer siempre en los momentos más determinantes y oportunos. Sin duda alguna, nuestra amistad es el mejor fruto cosechado durante mi labor como doctorando. Aunque cada año nos cuesta más esfuerzo, espero que sigamos regando semejante amistad que con tanto esmero hemos cultivado.

A Mikel, por su inagotable respuesta del *"Sí"*, por su empatía, compañerismo y generosidad, y como no, por desconocer el precio que se paga cuando no se intenta. Espero que ese precio te sea desconocido por muchos años más. Gracias.

Junto a Mikel, agradecer al resto de compis de la UPNA por recibirme con sonrisas amables y brazos abiertos el primer día, y por aconsejarme, apoyarme y escucharme el resto de los días. Gracias Miguel, Roberto, Arantza, Alazne, Mikel Jr., Iker,...

Agradecimientos inevitables por y para todos esos *Simples,* miembros del *C.D. Calvoruña* y el resto de asociados *calvos* que han formado junto a mí tanto en los campos de batalla confortables como en los insoportables. Este mundo paralelo me ha ayudado a mantener mi peculiar equilibrio desequilibrado.

A la *Crew*, David Delgado (el peso pesado) y al resto de *Viejas Glorias del Karate Navarro* (*VGKN*) por comprender lo incompresible y por no haber alejado el hombro que un día arrimasteis junto al mío. Arreando que es gerundio.

Gracias a Pitzi por ser capaz de romper el ritmo imperfecto marcado por el paso del tiempo. Nunca nadie había sido capaz de hacer esto por mí. Gracias.

Gracias a todos esos *canariones* comandados por José Antonio López Calbet por regalarme un año inolvidable. La experiencia concedida supuso un granito distinguido en la composición de toda esta historia aun sin terminar, pero a punto de culminar.

A pesar de haber sido en una etapa anterior a este doctorado, no puedo evitar acordarme de Geraint Florida-James, por haberme enseñado a aprender. Nunca lo olvidaré. Gracias.

Por todos esos co-currelas, amigos, compañeros, colaboradores y personajillos que se han cruzado en mi vida durante mi periodo doctorando. De manera directa, indirecta o más indirectamente, todos habéis contribuido a la elaboración del presente documento. La lista a los que agradecer sería interminable. Como ya sabéis que estáis en esta lista, permitirme ahorrar la imprenta de unos cuantos folios. Gracias.

Gracias a Braulio y Brutus, por quererme como soy

A quien no se olvida, no desaparece

Mil agradecimientos, (y aun me queda alguno más)

Ibai

Financial Support, List of Publications and Conference Papers

Financial Support

The main financial supporters of the current dissertation were the Studies, Research and Sports Medicine Center of the Government of Navarre and the Department of Health Sciences of the Public University of Navarre.

Awards and Scholarships

Educational Grant for the Formation in Research Personnel. Public University of Navarra
July 2015 - Currently

Navarre Scholarship 2012-2015 for the Formation in Sport and Exercise Sciences Research. Studies, Research and Sports Medicine Center, Navarre Sport and Exercise Institute, Government of Navarre
September 2012 - July 2015

Navarre Scholarship 2011-2012 for Sport and Exercise Science Licentiates. Studies, Research and Sports Medicine Center, Navarre Sport and Exercise Institute, Government of Navarre
September 2012 - July 2015

List of Publications

Llodio I, Gorostiaga EM, **Garcia-Tabar I**, Granados C, Sánchez-Medina L. Estimation of the maximal lactate steady state in endurance runners. *Int J Sports Med* 2016; Epub ahead of print

Garcia-Tabar I, Llodio I, Sánchez-Medina L, Asiain X., Ibañez J, Gorostiaga EM. Validity of a single lactate measure to predict fixed lactate thresholds in elite athletes. *J Sport Sci* 2016; Epub ahead of print

Garcia-Tabar I, Eclache JP, Aramendi JF, Gorostiaga EM. Gas analyzers' drift leads to systematic error in maximal oxygen uptake and maximal respiratory exchange ratio determination. *Front Physiol* 2015; 30(6):308.

Llodio I, **Garcia-Tabar I**, Sánchez-Medina L, Ibañez J, Gorostiaga EM. Estimation of the maximal lactate steady state in junior soccer players. *Int J Sports Med* 2015; 36(14):1142-1148.

Garcia-Tabar I, Llodio I., Sánchez-Medina L, Ruesta M, Ibañez J, Gorostiaga EM. Heart rate based prediction of fixed blood lactate thresholds in professional team-sport players. *J Strength Cond Res* 2015; 29(10):2794-2801.

Garcia-Tabar I, Sánchez-Medina L, Aramendi JF, Ruesta M, Ibañez J, Gorostiaga EM. Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists. *J Exerc Physiol Online* 2013; 16(5):38-50.

Conference Papers

Garcia-Tabar I, Gil Quintana E, Aguirre González A, Setuain I, Barrena Montalvo R, Leoz-Abaurrea I, Aguado-Jiménez R, Barajas MA, Izquierdo M. Eggshell membrane in the treatment of pain and stiffness associated with joint and connective tissue disorders. Results from a clinical pilot study in humans. ***Int J Sport Nutr Exerc Metab*** 2016; 26:S1-S15. ***International Sports and Exercise Nutrition Conference 2015***

Poster presentation by Garcia-Tabar I

15-17 December 2015, Newcastle, United Kingdom

Garcia-Tabar I, Gil Quintana E, Aguirre González A, Setuain I, Barrena Montalvo R, Leoz-Abaurrea I, Aguado-Jiménez R, Barajas MA, Izquierdo M. Suplementación con membrana de cáscara de huevo para tratar el dolor y la rigidez articular. ***Arch Med Deporte*** 2015; 32(5):314-334. ***VI Jornadas Nacionales de Medicina del Deporte***

Oral communication by Garcia-Tabar I

27-28 November 2015, Bilbao, Spain

Garcia-Tabar I, Asiain X, Setuain I, Gorostiaga EM. A practical alternative to fixed blood lactate thresholds in athletes. ***20th anual Congress of the European College of Sport Science.*** ISBN 978-91-7104-567-6

Oral communication by Garcia-Tabar I

24-27 June 2015, Malmö, Sweden

Setuain I, Bikandi E, Idoate F, Izquierdo M, **Garcia-Tabar I**, Alfaro-Adrián J. Radiological study of changes in muscle volume using MR imaging in patients undergoing ACL reconstruction of the knee with semitendinosus and gracilis autograft. ***20th anual Congress of the European College of Sport Science .***ISBN 978-91-7104-567-6

Oral communication by Setuain I

24-27 June 2015, Malmö, Sweden

Garcia-Tabar I, Llodio I, Setuain I, Sánchez-Medina L, Ruesta M, Ibañez J, Gorostiaga EM. Heart rate predicts anaerobic lactate thresholds in professional team sports. ***19th anual Congress of the European College of Sport Science.*** ISBN 978-94-622-8477-7

Oral communication by Garcia-Tabar I

2-7 July 2014, Amsterdam, Netherlands

Perez-Valera M, Morales-Álamo D, Rodriguez-Noda A, Torres-Peralta R, Losa-Reyna J, Perez-Suarez I, De La Calle Herrero J, **Garcia-Tabar I**, Cuertelin D, Calbet JA. Cerebral oxygenation during repeated wingate test. ***19th anual Congress of the European College of Sport Science.*** ISBN 978-94-622-8477-7

Oral communication by Perez-Valera M

2-7 July 2014, Amsterdam, Netherlands

Setuain I, Millor N, Gorostiaga EM, **Garcia-Tabar I,** Alfaro J, González-Izal M, Izquierdo M. Jumping acelerometric study of elite handball female athletes with or without previous anterior cruciate ligament reconstruction. ***19th anual Congress of the European College of Sport Science.*** ISBN 978-94-622-8477-7

Oral communication by Setuain I

2-7 July 2014, Amsterdam, Netherlands

Grijalba A, Martín J, **Garcia-Tabar I**, Rivero-Marcotegui A, Sánchez-Medina L, Gorostiaga EM. Efectos del entrenamiento de fuerza con pesas en el salto vertical y el balance hormonal en futbolistas jóvenes. ***VII Congreso Nacional del Laboratorio Clínico.***

Poster presentation by Grijalba A

26-29 November 2013, Bilbao, Spain

Garcia-Tabar I, Sánchez-Medina L, Aramendi JF, Ruesta M, Ibañez J, Gorostiaga EM. Heart Rate Variability Thresholds Predict Lactate Thresholds in Professional World-Class Road Cyclists. ***18th annual Congress of the European College of Sport Science.*** ISBN 978-84-695-7786-8

Oral communication by Garcia-Tabar I

26-29 June 2013, Barcelona, Spain

Summary (English)

Resumen (Castellano)

Summary

The current Ph.D. dissertation revolves around the determination of aerobic capacity, with special reference to the development of practical strategies to overcome some limitations that sport scientific practitioners usually encounter at the time to give scientific endurance support to athletes or physical activity practitioners. This doctoral thesis is based on 4 scientific studies that have been published or accepted for publication in scientific international journals. The first study (Chapter 2) is a methodological laboratory-based study concerning one of the most classical and fundamental measurements in exercise physiology; the maximal oxygen uptake. Studies 2, 3 and 4 (Chapters 3, 4 and 5) are field-based studies carried out during regular sport scientific support given to elite and professional athletes.

Study 1 (Chapter 2)

Maximal oxygen uptake is nowadays usually measured with automated metabolic systems. These automated systems may include errors related to the instability of the gas analyzers, which are prone to drift over time. It is generally assume that gas analyzing systems are stable throughout the exercise and that respiratory measures are valid as long as pre-test calibration procedures are followed. Clearly, the process of after trial verification tends to be overlooked and has the potential to introduce relevant errors into the accuracy of maximal respiratory measurements. The aim of this first study was to examine the drift in the measurements of fractional concentrations of oxygen and carbon dioxide of a Nafion-using metabolic cart during incremental maximal exercise in 18 young and 12 elderly males. The drift was verified by comparing the pre-test calibration values with the immediate post-test verification values of the calibration gases. We found a significant and systematic downscale drift from pre-test to post-test readings of oxygen and carbon dioxide, resulting in an average maximal oxygen uptake overestimation of 5-6%. The drift was not due to an electronic instability in the analyzers because it was reverted after 20 minutes of recovery from the end of the exercise. The drift may be related to an incomplete removal of water vapor from the expired gas during transit through the Nafion conducting tube. We consider that the error found is not acceptable to test athletes, to prescribe exercise intensities, or to use the respiratory values for some other clinical purposes, such as to guide treatment in patients with chronic heart failure, to enter in cardiac transplantation listing, to indicate the health status or to predict prognosis and mortality. We therefore proposed a correction method to reduce the error associated with the drift. These findings are original and emphasize the need for an adequate initial calibration of the analyzers, but also the need for a post-test verification and further correction of the drift in order to determine adequately subjects' maximal oxygen uptake.

Studies 2, 3 and 4 (Chapters 3, 4 and 5)

Lactate thresholds measured during a single graded incremental exercise test are aerobic capacity markers broadly use among sport scientists to assess the physiological capacity of athletes and prescribe endurance training. The determination of lactate thresholds, however, requires qualified personnel for invasive blood sampling. This often hinders frequent (weekly or monthly) lactate thresholds' determination as would be required for ongoing monitoring of the aerobic capacity and proper adjustments in endurance training intensity. As a result, determination of

lactate thresholds is often only conducted 2-3 times per season in granted or professional teams and athletes, while the majority of the recreational and club level athletes do not have the opportunity to determine their lactate thresholds. Therefore, the primary aim of the 3 next studies was to scientifically evaluate alternative methods for lactate thresholds' determination requiring minimal equipment in order to be able to assess and monitor endurance capacity more frequently.

Study 2 (Chapter 3)

The aim of the second study was to investigate whether the speed associated with 90% of maximal heart rate could predict the speeds associated with fixed blood lactate concentrations of 3 mmol·L^{-1} and 4 mmol·L^{-1}. Professional team-sport players of futsal (n = 10), handball (n = 16) and basketball (n = 10) performed a four-stage discontinuous progressive running test followed, if exhaustion was not previously achieved, by an additional maximal continuous incremental running test to attain maximal heart rate. The individual fixed blood lactate concentration thresholds and the speed associated with 90% of maximal heart rate were determined by linear interpolation. The individual fixed blood lactate concentration thresholds did not differ from the speed associated with 90% of maximal heart rate, and there were very large relationships between them. It was demonstrated that the fixed blood lactate concentration thresholds can be accurately predicted by the speed associated with 90% of maximal heart rate in professional team-sport players. The use of the speed at 90% of maximal heart rate would cheapen and facilitate endurance performance's assessment through a non-invasive and surprisingly easy method. We strongly think that this finding could be implemented in the field and be really helpful for athletes and coaches with limited resources.

Study 3 (Chapter 4)

In the third study we proposed three new mathematical determined heart rate variability thresholds to estimate the lactate thresholds. Twelve male professional world-class road cyclists performed a continuous maximal graded cycling test. Blood lactate concentration, heart rate and RR intervals were monitored. Four different lactate thresholds (1 aerobic and 3 anaerobic lactate thresholds) were determined. Heart rate variability thresholds were determined from the standard deviation of the instantaneous beat-to-beat RR intervals. Large to very large relationships were found between the lactate thresholds and the heart rate variability thresholds. Results indicated that lactate thresholds can be accurately predicted from values obtained with a heart rate monitor during a maximal or submaximal, non-invasive, low-cost, incremental exercise test measurable in non-laboratory settings without the need of any technical expertise to administer the test.

Study 4 (Chapter 5)

The last study of the current Ph.D. dissertation validated the use of a single blood lactate concentration measure taken following a 12 km·h^{-1} running stage to predict and monitor fixed blood lactate concentration thresholds. Three complementary studies were undertaken. *Study I*: the relationships between the blood lactate concentration at 12 km·h^{-1} and the running speeds at fixed blood lactate concentrations of 3 mmol·L^{-1} and 4 mmol·L^{-1} measured during a multistage running field test were examined in 136 elite athletes. *Study II*: data from 30 athletes tested one

year apart were used to test the predictive capacity of the equations obtained in *Study I. Study III*: 80 athletes were tested before and after an intensified training period to examine whether training-induced changes in fixed blood lactate concentration thresholds could be predicted and monitored by a single lactate measure taken at 12 $km \cdot h^{-1}$. *Study I*: the blood lactate taken at 12 $km \cdot h^{-1}$ was close related to both fixed blood lactate concentration thresholds. *Study II:* prediction models yielded robust correlations between the estimated and measured fixed blood lactate thresholds. *Study III*: estimated changes predicted actual training-induced changes in fixed lactate thresholds. This study gives empirical support to use a single lactate measure during a sub-maximal running field test as a simple, low-cost and practical alternative to fixed lactate thresholds in athletes. Results of these three complementary studies could be interesting for teams, clubs and athletes with limited resources.

Resumen

La presente tesis doctoral gira entorno a la determinación de la capacidad aeróbica, con especial énfasis en tratar de solventar algunas de las limitaciones que los científicos y profesionales del ejercicio físico y del deporte nos encontramos a la hora de asistir a deportistas o poblaciones especiales que quieren (o necesitan) practicar ejercicio físico de manera adecuada. Para ello se han llevado a cabo 4 estudios de investigación, 3 estudios basados en la asistencia científica real que ofrecemos de manera regular a deportistas profesionales o de élite que tienen algún tipo de financiación económica (capítulos 3, 4 y 5) y otro estudio algo más experimental en deportistas amateurs e individuos de edad avanzada (capítulo 2).

Estudio 1 (Capítulo 2)

Tradicionalmente la capacidad aeróbica se ha determinado mediante la medición del consumo máximo de oxígeno. Hoy por hoy, el consumo máximo de oxígeno se mide mediante sistemas metabólicos automáticos. Estos sistemas automáticos pueden tener errores relacionados con la inestabilidad de los analizadores de gases, ya que los analizadores de gases son propensos a tener cierta deriva con el transcurso del tiempo. A pesar de ello, por lo general, la comunidad científica asume que los analizadores de gases son estables durante los teses de ejercicio, y que las variables respiratorias medidas con los analizadores de gases son válidas siempre y cuando se haya realizado una calibración previa al test de ejercicio. De acuerdo con la literatura, parece ser que la mayoría de los profesionales del ámbito del ejercicio físico y del deporte realizan una calibración previa a los teses de ejercicio, pero en cambio, habitualmente, la verificación después de los teses de ejercicio se ignora. Ignorar la verificación post-ejercicio tiene el potencial de inducir cierto error de medida en la determinación del consumo máximo de oxígeno, tal y como demostramos en el primer estudio de la presente tesis. En este primer estudio (Capítulo 2) nuestro objetivo fue examinar la deriva de nuestro analizador de gases durante un ejercicio incremental hasta el agotamiento en 18 deportistas amateurs y 12 individuos de edad avanzada. Encontramos una deriva sistemática en las en las concentraciones de oxígeno y dióxido de carbono que a su vez provocaron una sobrestimación en la determinación del consumo máximo de oxigeno del 5-6%. A nuestro juicio, este error es demasiado grande para poder evaluar la capacidad aeróbica y con ello prescribir ejercicio físico en deportistas o en poblaciones especiales, para indicar la salud de los pacientes, predecir la mortalidad, o para usar el consumo máximo de oxígeno como criterio para entrar las listas de trasplantes. Por ello, en este mismo estudio, proponemos un método de corrección para reducir el error asociado con la deriva sistemática que hemos encontrado en nuestro analizador de gases. Los hallazgos de este estudio son originales y enfatizan la necesidad de calibrar los analizadores de gases antes de iniciar los teses de ejercicio, pero también enfatizan la necesidad de verificar los valores medidos nada más acabar los teses de ejercicio. Con el proceso de verificación se pueden corregir los errores inducidos por la deriva, y de esa manera, determinar de una manera más precisa la capacidad aeróbica.

Estudios 2, 3 y 4 (Capítulos 3, 4 y 5)

Los umbrales de lactato medidos durante un test de ejercicio incremental son indicadores de la capacidad aeróbica que los científicos del ejercicio físico y del deporte usamos con mucha

frecuencia para evaluar la condición física de los deportistas y pacientes, y con ello poder prescribir ejercicio físico de resistencia. El mayor inconveniente a la hora de determinar los umbrales de lactato es que se requiere personal cualificado para obtener las muestras de sangre, lo que impide su uso frecuente para monitorizar la capacidad aeróbica como es debido. A los equipos, clubes o deportistas con algún tipo de financiación se les determinan los umbrales de lactato 2-3 veces por temporada, mientras que la mayoría de los atletas recreacionales no tienen acceso a este tipo de evaluaciones. Por eso, en los siguientes 3 estudios de esta tesis, el propósito principal fue tratar de hallar alternativas o herramientas útiles para estimar los umbrales de lactato de una manera más práctica, sencilla y barata, y de ese modo ser capaces de evaluar y monitorizar la condición aeróbica con mayor frecuencia.

Estudio 2 (Capítulo 3)

El objetivo del segundo estudio fue investigar si la velocidad asociada con un determinado porcentaje de la frecuencia cardiaca era capaz de estimar los umbrales de concentraciones fijas de lactato de 3 y 4 $mmol \cdot L^{-1}$. En el estudio participaron 3 equipos profesionales de futbol sala (n = 10), balonmano (n = 16) y baloncesto (n = 10). Los participantes realizaron una prueba discontinua incremental para la determinación de los umbrales de concentraciones fijas de lactato, y se estudió la relación de los umbrales de lactato con la velocidad asociada al 90% de la frecuencia cardíaca máxima. La velocidad asociada al 90% de la frecuencia cardíaca máxima no fue diferente a las velocidades asociadas a los umbrales de concentraciones fijas de lactato, y mostró una estrecha relación con ambos umbrales. Este estudio demostró que los umbrales de concentraciones fijas de lactato se pueden estimar de manera precisa usando tan solo un pulsómetro en deportistas profesionales de deportes de equipo. El uso del 90% de la frecuencia cardíaca máxima puede facilitar la monitorización de la capacidad aeróbica mediante un método no-invasivo y sorprendentemente fácil de implementar en el campo, por lo que puede servir de gran ayuda a deportistas y entrenadores con recursos limitados.

Estudio 3 (Capítulo 4)

En el tercer estudio se proponen 3 nuevos métodos matemáticos para determinar los umbrales de la variabilidad de la frecuencia cardiaca con el fin de estimar los umbrales de lactato. Doce ciclistas profesionales de categoría mundial realizaron un test continuo máximo incremental hasta el agotamiento para la determinación de los umbrales de lactato. Durante la prueba incremental se midió el lactato sanguíneo, la frecuencia cardiaca y el intervalo de tiempo entre latido y latido. Se determinaron 4 umbrales de lactato (1 aeróbico y 3 anaeróbicos). También se determinaron 3 umbrales de la variabilidad de la frecuencia cardiaca usando el eje transversal de la desviación estándar de los intervalos RR en los gráficos de dispersión de Poincaré. La intensidad asociada al umbral aeróbico de lactato fue similar a las intensidades asociadas a los umbrales de la variabilidad de la frecuencia cardiaca. Se observaron unas estrechas relaciones entre los umbrales de la variabilidad de la frecuencia cardiaca y los umbrales de lactato. Los resultados de este estudio demuestran que los umbrales de lactato pueden ser estimados de manera precisa mediante la variabilidad de la frecuencia cardiaca durante un protocolo de ejercicio sub-máximo o máximo, incruento y barato en ciclistas profesionales de categoría mundial.

Estudio 4 (Capítulo 5)

El último estudio de la presente tesis tuvo como objetivo validar el uso de una sola toma de lactato tomada a 12 $km \cdot h^{-1}$ después de un test sub-máximo de 13 minutos para predecir y monitorizar los umbrales de concentraciones fijas de lactato. Para ello se llevaron a cabo 3 estudios complementarios. *Estudio I*: se estudiaron las relaciones entre la toma de lactato a 12 $km \cdot h^{-1}$ y los umbrales de concentraciones fijas de 3 y 4 $mmol \cdot L^{-1}$ determinados mediante un test de ejercicio discontinuo incremental en 136 deportistas de élite. *Estudio II*: los datos de 30 deportistas evaluados un año después se usaron para valorar la capacidad predictiva de las ecuaciones obtenidas en el *Estudio I*. *Estudio III*: 80 deportistas fueron evaluados antes y después de un periodo intensificado de entrenamiento para examinar si los cambios en los umbrales de lactato inducidos por el entrenamiento se podían predecir y monitorizar usando la toma de lactato a 12 $km \cdot h^{-1}$. *Estudio I:* el lactato a 12 $km \cdot h^{-1}$ y los umbrales de concentraciones fijas de lactato estaban estrechamente relacionados. *Estudio II:* usando los modelos predictivos desarrollados en el *Estudio I* se hallaron unas relaciones muy estrechas entre los umbrales de lactato estimados y medidos. *Estudio III:* los cambios en los umbrales de lactato estimados mediante el lactato a 12 $km \cdot h^{-1}$ estaban estrechamente relacionados con los cambios reales. Este estudio demuestra que el uso de una sola toma de lactato medida durante un test de campo sub-máximo de tan solo 13 minutos puede ser útil para predecir y monitorizar los umbrales de concentraciones fijas de lactato de una manera sencilla, barata, práctica y precisa. Estos hallazgos pueden ser útiles y muy interesantes para equipos y clubes deportivos con limitados recursos económicos.

Chapter 1

A brief historical remark on aerobic capacity determination

An Introduction

A brief historical remark on aerobic capacity determination. An Introduction

Efforts to understand human physiology and to model aerobic capacity or endurance performance have been ongoing for about a century (Bassett, 2002; Hale, 2008). Previous authors, in the introduction section of exercise physiology textbooks (Calbet, 2006; McArdle, Katch, & Katch, 2001), have wonderfully synthesized the wide history of the development of modern aerobic capacity determination (modern endurance assessment and testing), and it would be too difficult to improve on their efforts. Instead, this section will attempt to summarize the most important contributions and place them in a historical perspective by viewing them in the context of the findings of the present dissertation.

Modern aerobic capacity testing has been supplied by 2 historical research pathways: 1) the use of exercising athletes to better understand human physiology and 2) the use of human physiology to better understand how to train and test athletes. Together these two research pathways have led some of the world's most recognized exercise physiologists to develop the endurance capacity model which is nowadays well accepted in the international exercise physiology community. This model describes the aerobic capacity or endurance performance to be determined by the integration of 3 main factors that are 1) maximal oxygen uptake ($\dot{V}o_{2max}$), 2) fractional utilization of $\dot{V}o_{2max}$ at the lactate threshold (LT) or at the maximal lactate steady state (MLSS), and 3) work economy or skeletal muscle efficiency (Bassett & Howley, 1997; Bassett & Howley, 2000; Coyle, 1995; Joyner, 1991; Joyner & Coyle, 2008; Lundby & Robach, 2015) (Figure 1). It is to note that this model vary a little bit depending upon the physiologists. According to some of them (Bassett & Howley, 1997; Bassett & Howley, 2000) the workload at the LT or MLSS integrates all the three core variables abovementioned and best predicts endurance performance. Instead, other authors (Coyle, 1995; Joyner & Coyle, 2008; Lundby & Robach, 2015; Weltman 1995) consider that the second key determinant of aerobic capacity is not the fractional utilization of $\dot{V}o_{2max}$ at the LT or MLSS, but the LT or MLSS itself. Anyhow, most of the laboratories around the world performing physiological testing on endurance athletes routinely measure $\dot{V}o_{2max}$, LT or MLSS (or estimations of MLSS), and, to a lesser extent, work efficiency.

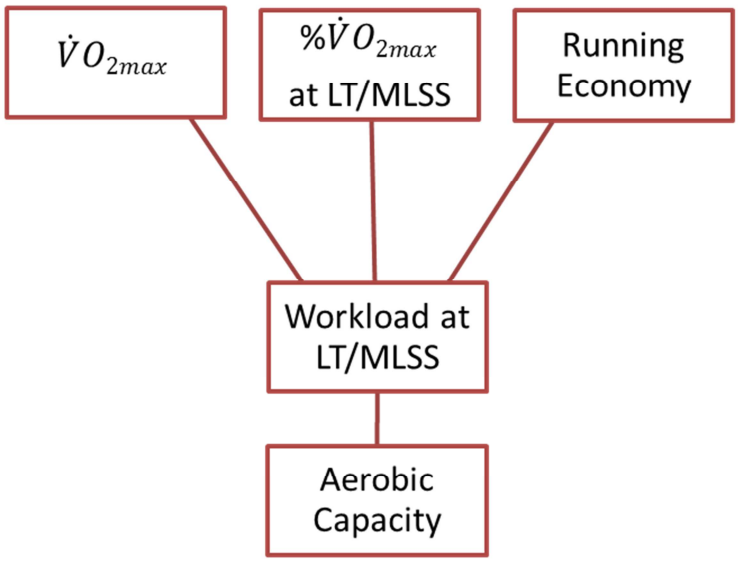

Figure 1: Simplified diagram from Bassett and Howley (1997; 2000) of mayor determinants of aerobic capacity

In the particular case of our team of sport scientific practitioners, LT and MLSS, or MLSS estimations, are routinely assessed in laboratory and field settings as part of the regular longitudinal monitoring of the aerobic capacity of our endurance athletes. $\dot{V}o_{2max}$ is also routinely measured, but, in this case, as part of laboratory-based research projects. The current Ph.D. dissertation, therefore, revolves around the determination of aerobic capacity by the first two core variables ($\dot{V}o_{2max}$ and LT or MLSS), with special reference to the development of practical strategies to overcome some limitations that sport scientific practitioners usually encounter at the time to give scientific endurance support to athletes or physical activity practitioners.

A brief history of the maximal oxygen uptake

Maximal oxygen uptake ($\dot{V}o_{2max}$) is one of the most fundamental measures of exercise physiology. Certainly, it has been considered "*the single most influential concept in modern exercise physiology*" (Noakes, 1998), and the first component of the endurance performance model to be discovered (Bassett & Howley, 1997; Hawkins, Raven, Snell, Stray-Gundersen, & Levine, 2007; Levine, 2008). $\dot{V}o_{2max}$ is defined as the physiological upper limit of the body's ability to consume and utilize oxygen per time unit, and it is usually measured during incremental large muscle mass exercise to exhaustion during which respiratory gases are analyzed.

Records in respiratory physiology date back to the 1770s. Knowledge concerning the role that oxygen plays in combustion arose with the experiments conducted by the "*father of modern chemistry*", the French Antoine Lavoisier (1743-1794), although his experiments were documented by his wife Marie-Anne Pierrette Paulze (Duveen & Klickstein, 1955). Due to the similarities between combustion and respiration, Lavoisier extended his theory of combustion to the area of respiratory physiology. He is considered the first investigator to carry out research studies on human respiration. Lavoisier concluded in one of his investigations that "*la respiration est donc une combustion*" (Buchholz & Schoeller, 2004). His beheading came in 1794.

Since then, many investigators have made substantial contributions to the evolution of exercise physiology and to the current approach of endurance testing. Just to name a few, William Prout (1785-1850), an Englishman from Gloucestershire, measured carbon dioxide exhaled by men exercising to fatigue and observed the carbon dioxide plateau phenomenon during submaximal exercise. These observations heralded the modern concept of steady-state gas exchange kinetics in exercise (McArdle et al., 2001). Edward Hitchcock father (1793-1864) and Edward Hitchcock son (1828-1911) pioneered the sports science movement in the States. Their contributions were essential for the foundation of the first formal exercise physiology laboratory in the United States, which was established in 1891 at Harvard University. In Germany, Eduard Friedrich Wilhelm Pflüger (1829-1910) founded the foregoing journal of the *Pflügers Archiv - European Journal of Physiology,* and provided training to a group of researches conducting innovative research on exercise physiology. This research group, commanded by Nathan Zuntz (1847-1920), opened the first sports medicine laboratory in Germany and constructed in 1889 an early treadmill named "*Laufband*". Figure 2 depicts the great challenge it was at the time to measure physiological responses at a given known workload in exercising humans under laboratory conditions. The drawing illustrates the "*fatigometer*" developed in the Harvard Fatigue Laboratory between 1927 and 1947. Close in time to the "*Laufband*", John Scott Haldane (1860-1936), a Scottish worldwide

recognized authority on respiration (and the originator of the gas-mask during World War I), improved the measuring methods of the fractional concentrations of expired oxygen (F_EO_2) and carbon dioxide (F_ECO_2) by pyrogallol and potassium hydroxide absorbing agents. At the beginning of the 20th century, Tissot developed a device (spirometer) to measure humans' respiration. At about the same time, an English physiologist born in Leicester, Claude Gordon Douglas (1882–1963), described the Douglas bag method for collection of expired air and analyses of oxygen uptake ($\dot{V}o_2$) and carbon dioxide output ($\dot{V}co_2$) (Douglas, 1911). But it was not till 1923 that came the birth of $\dot{V}o_{2max}$.

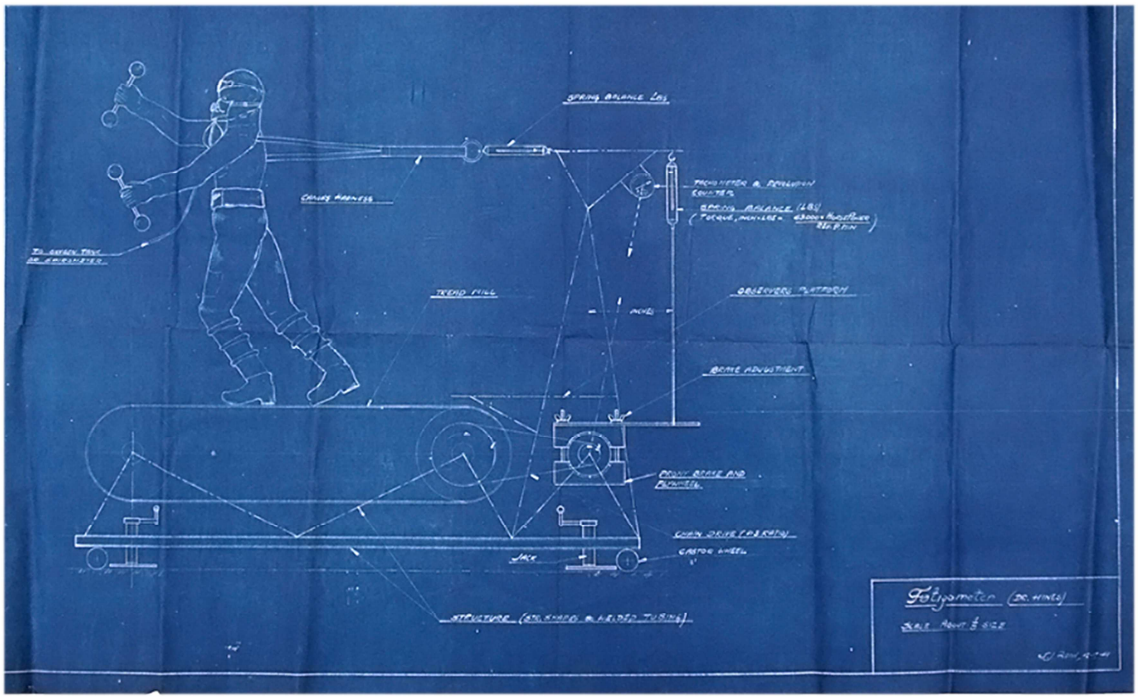

Figure 2: This drawing from the Harvard Fatigue Laboratory illustrates the "*fatigometer*". The drawing was retrieved the 13th of May 2016 from http://www.survivalofthefitness.info/brief-history-of-exercise-physiology/

Historical literature in the field of respiratory physiology recognize (Bassett, 2002; Hale, 2008; Hawkins et al., 2007; Levine, 2008; Noakes, 1988) Novel laureate Archibald Vivian (AV) Hill (1886-1977) to be the first English-speaking scientist to study in detail the oxygen consumption during exercise, and to be the inventor of the concept of "*maximal oxygen intake*" and oxygen uptake plateau in 1923 (Hill & Lupton, 1923). Some years earlier, AV Hill completed careful investigations on the heat production on contracting skeletal muscles of frogs. During the same time, parallel to Hill's studies, Otto Meyerhof (1884-1951) studied muscle glycogen and lactate changes during contractions and recovery. The discovery of the distinction between aerobic and anaerobic metabolisms on their muscle metabolism studies brought them to share the 1922 Noble Prize. Because of Hill's attraction to athletics, he endeavored to apply the discoveries in isolated frog muscle to exercising humans. Hill was the first researcher to report the linear relationship between $\dot{V}o_2$ and workload in 1923-1925 (Hill & Lupton, 1923; Hill, Long, & Lupton, 1924a; Hill, Long, & Lupton, 1924b). The experimental protocol used by Hill was a discontinuous incremental

test that consisted on running 3 min at the speeds of 10.3, 11.9, 14.6, and 16.1 km·h^{-1} on an open-air 85-m circular grass track in Manchester. The expired air samples were collected by Douglas bags every 30 sec, gas concentrations were measured by a Haldane gas analyzer and the bag volume by means of a Tissot gasometer. It is important to recall that at the time, the $\dot{V}o_2$-workload relationship was constructed with data accumulated over several days because a single running speed was tested per day. It took several decades until $\dot{V}o_{2max}$ testing became relatively practical. In spite of the limitations at the time, data reported by Hill and colleagues were demonstrated to be remarkably accurate (Noakes, 1988) by comparing them with data collected more than half of a century later (Leger & Mercier, 1984). Furthermore, Hill and partners not only described the concept of $\dot{V}o_{2max}$, but attempted to explain its physiological mechanisms. Interpretations and conclusions drawn by Hill and collaborators were well accepted by exercise physiologists for the next ≈70 years. Relatively recently, the concept of $\dot{V}o_{2max}$ was critically evaluated, and a lively debate about the limiting factors in $\dot{V}o_2$ emerged. The most serious critics were initiated by the South African Timothy D. Noakes (Noakes, 1988; Noakes, 1997; Noakes, 1998; Noakes, Peltonen, & Rusko, 2001; Noakes & Marino, 2009), and provoked resounding rebuttals from some other worldwide physiologist such as Bassett and Howley (1997), Bergh, Ekblom, and Åstrand (2000) and Robert A. Robergs (2001). This debate indicates, beyond a shadow of a doubt, that knowledge concerning $\dot{V}o_{2max}$ provided by Hill and his co-scientists is still fundamental to our understanding of endurance performance limitations today. Hill's work served to legitimize the field of exercise physiology as a new scientific discipline (Bassett, 2002).

Standardization of $\dot{V}o_{2max}$ testing arrived with the careful experimental studies done by Henry Longstreet Taylor in the 1950s, and Per-Olof Åstrand and Bengt Saltin in the 1960s. During World War II, Taylor and coworkers performed several laboratory-based studies in the Laboratory of Physiological Hygiene at the University of Minnesota in the United States. In mid 1950s Taylor et al. (1955) published a classical paper that, according to Hale (2008), established the standard methodological procedures for $\dot{V}o_{2max}$ testing for decades. Taylor's participants were mostly military soldiers who were conscientious objectors. Subjects pretty much lived and trained following protocols set up by Taylor and colleagues. The fact that Taylor's 12 subjects exercised for an hour per day, 6 days a week during a whole year, clearly shows soldiers disposal for laboratory issues and makes those experiments to remain unsurpassed today. Investigation of $\dot{V}o_{2max}$ as an aerobic capacity determinant reached its peak during the 1960s with experiments carried out by Åstrand and his first Ph.D. student Saltin, both affiliated to the Swedish Sport University and Karolinska Institute in Sweden. They published a large amount of normative data for a wide range of sports showing a clear relationship between endurance performance and $\dot{V}o_{2max}$ (Astrand & Saltin, 1961a; Astrand & Saltin, 1961b; Saltin & Astrand, 1967).

The discontinuous test protocols utilized by Hill and Taylor, and sometimes Åstrand, were extended over days or weeks, and were therefore claimed to be impractical, and far from consolidation and standardization for clinical use (Mitchell, Sproule & Chapman, 1958). As far as I concern, Mitchell, Sproule and Chapman (1958) were some of the first investigators in reducing the time of $\dot{V}o_{2max}$ determination to a single day using 10-15 min intervals between stages rather than days. Popularization of ramp test protocols, however, did not arrive till the publication of the influential and painstaking experimental studies of Karlman Wasserman and his co-investigators (Beaver, Wasserman, & Whipp, 1973; Wasserman, Whipp, Koyl, & Beaver, 1973;

Whipp, Davis, Torres, & Wasserman, 1981). Wasserman is best known among exercise physiologists for his research on the determination of aerobic and anaerobic thresholds. Wasserman was trying to determine blood lactate thresholds (LTs) by indirect respiratory gas exchange measurements when he joined William Beaver. As a result of this cooperation between public academic and private industry worlds, Wasserman and Beaver developed the necessary technology for the breath by breath gas exchange sampling approach. It is believed that the capability of Beaver to develop digital computing interfaces was crucial to put Wasserman's theory into practice and achieve such a repercussion. Up to the late 1960s and early 1970s, gas exchange related measurements were undertaken in controlled laboratory conditions via open-circuit indirect calorimetry using Douglas bags, respiratory gasometers, and chemical analysers. During the 1960s and 1970s, the development of rapidly responding electronic analysers coupled with electronic activated gas volume-sensing devices and computers, were a key advance in the development of automated systems for assessing ventilatory function during exercise (Beaver et al., 1973; Kissen & McGuire, 1967).

Nowadays $\dot{V}o_{2max}$ is usually measured with automated metabolic systems. These automatic systems seem theoretically simple to use, but, the practical aspects related to their technology are difficult to implement with precision due to multiple errors that are difficult to control and detect (Gore, Tanner, Fuller, & Stanef, 2013; Howley, Bassett, & Welch, 1995). These errors might be related to the delay times between analysers, calibration procedures of the flow sensors, linearity and calibration of the gas analysers, the handling of water vapour before the gas enters the analyser and the fine tuning of the temporal alignment between ventilation and gas measurements (Atkinson, Davison, & Nevill, 2005). It is generally assume that respiratory measures are valid as long as pre-test calibration procedures are followed, and clearly, the process of after trial verification tends to be overlooked. Due to the reason that gas analyzers are prone to drift over time (Gore et al., 2013; Winter, 2012), omission of post-test verifications has the potential to introduce relevant errors into $\dot{V}o_{2max}$ determination. The first paper of the current Ph.D. dissertation (Chapter 2) examines the drift in the measurements of fractional concentrations of oxygen (FO_2) and carbon dioxide (FCO_2) during incremental exercise to exhaustion, and determines the influence of the drift on the determination of maximal respiratory measurements, such as $\dot{V}o_{2max}$. A correction method to reduce the error in $\dot{V}o_{2max}$ determination is also proposed. We hope that our big effort to provide a deep thoughtful discussion throws some light onto the understanding of the importance of after trials verifications when using automated "*black boxes*" (Figure 3) for $\dot{V}o_{2max}$ testing in this new century.

A brief history of the development of the lactate threshold concept

There is perhaps no theme in sports and exercise science that has been more discussed than the dealing with our understanding of lactate production and removal during exercise. Exercise and sports scientists have historically looked for a determinable individual critical point during incremental exercise to discern submaximal sustainable exercise intensity from intense fatiguing unsustainable exercise intensity. Once again AV Hill was one the first scientists to report the ventilatory turning point. Hill reported (Hill et al., 1924b) some ventilatory data collected during his experiments using the incremental discontinuous exercise test protocol abovementioned, where, according to Noakes (1988), a clear ventilation turnpoint at 14.5 $km \cdot h^{-1}$ can be clearly identified. However, Hill and co-scientists did not describe this phenomenon in detail. During that

time, Hill linked lactate production to muscle contraction, and his co-Novel Prize winner Meyerhoff related the increase in lactate concentration with muscle fatigue. Hill also noted that lactate increases with increasing exercise intensity (Hill et al., 1924a), which was subsequently corroborated by at least 10 other papers from other investigators before 1930 (Jones & Ehrsam, 1982). But this time, historical literature (Graham, 1984; Hale, 2008; Jones & Ehrsam, 1982; Noakes, 1988) in exercise physiology attributes another British scientist, Owles (1930), as the originator of the lactate threshold (LT) concept.

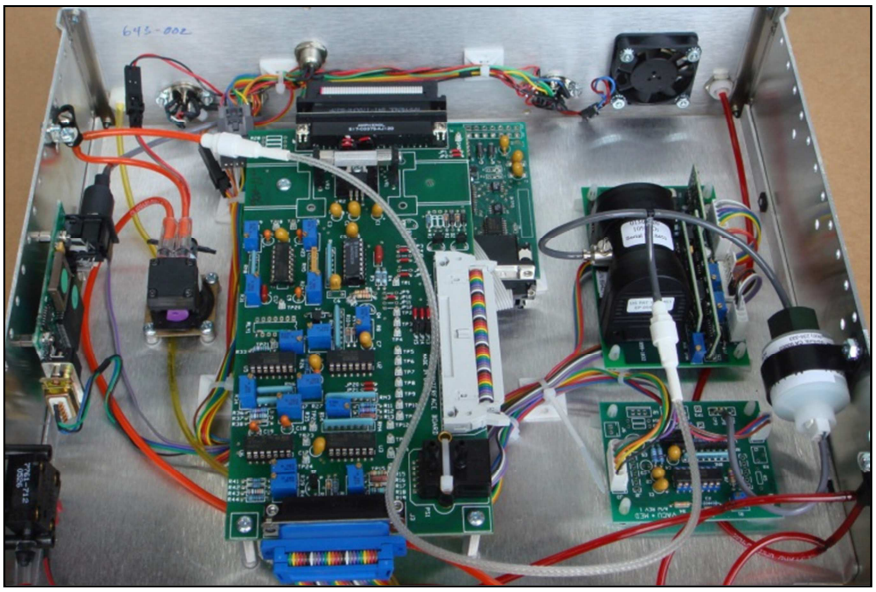

Figure 3: The inners of our laboratory's "*black box*" (Vista Mini-CPX, Vacu-Med, Silver Edition 17670)

The British scientist W. Harding Owles, working with C.G. Douglas at the Biomechanical and Physiological Laboratory at Oxford, first described a critical exercise intensity point (1930) based on blood lactate concentration (BLC) measures in 2 subjects (probably themselves, intuited by the initials of the subjects W.H.O. and C.G.D.) taken before and at the end of 14-40 min constant walking and cycling exercise intensities undertaken in separate days. Owles described that there was a critical exercise intensity level unique to each individual above which BLC progressively increased above resting values, later on referred as the "*Owles' Point*" (Jones & Ehrsam, 1982). Figure 4 shows BLC data after 14-40 min at different walking constant speeds collected on Owles, and reported by himself in the 7th table of his paper (1930). Regarding these data, Owles interpreted that "*Between 4.5 mph* (7.2 km·h^{-1}) *and 4.7 mph* (7.6 km·h^{-1}) *there seems to be……some critical rate of walk, above which only did an increase in blood lactate follow the exercise*" (1930, pp. 230), and follows, "*….there was not lactate increase as a result of exercise up to a certain critical level. This critical level varied in one individual for different types of exercise, and with training, and differed in different individuals……for walking exercise, this critical level corresponded to an oxygen utilization of about 1.8 L per min*" (1930 pp 234).

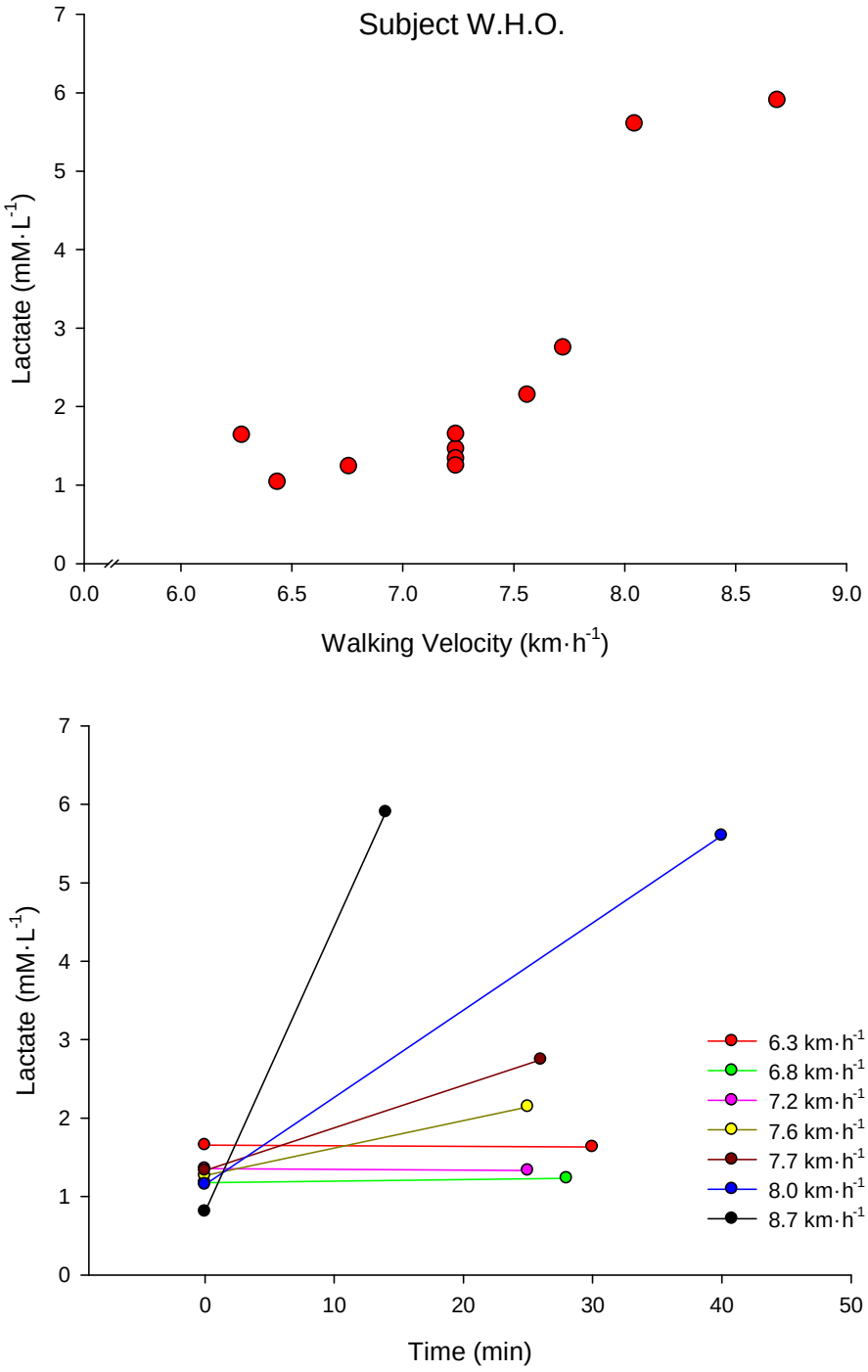

Figure 4: Lactate values of one subject reported by Owles (1930) illustrated as a function of velocity (upper panel) and time (lower panel). Data shows a critical intensity, around 7.2-7.6 km·h^{-1}, above which lactate increased above resting values, the "*Owles' Point*"

However, detail description of the lactate (LT) and ventilatory (VT) thresholds phenomena during incremental exercise (not during constant exercise workload tests) and methods to identify these thresholds probably began after the World War II, with the work presented at the 3rd Pan-American congress of Sports Physicians hold in Chicago (USA) in 1959 by Wildor Hollman, from the Cardiology and Sports Medicine Department of the German Sports University of Cologne.

According to this scientist (Hollmann, 1985), he was the one introducing the concept of the onset of anaerobic metabolism between the years of 1957 and 1963. Hollman explained in his retrospective LT-VT historical reviews (1985; 2001) that he found an identifiable breakpoint in the arterial BLC-workload curve during incremental exercise that represented individuals' aerobic performance capacity. At the time, there was no enzymatic micro-assay method for measuring BLC from capillary blood samples. Blood samples for measuring BLC were usually obtained by puncture of the arteria brachialis, and therefore, a thirst to noninvasively determine the standard arterial BLC increment (LT) (Beaver, Wasserman, & Whipp, 1985) during incremental exercise began. Hollmann (1985) explained how they commenced looking for ventilatory data to see whether they could identify a ventilatory breakpoint to estimate the arterial LT. According to him, they described the point at which minute ventilation increases in a greater rate than oxygen uptake. Hollmann named this breakpoint the "*point of optimum ventilatory efficiency*". Unfortunately Hollman findings were not published in English, and probably, he did not choose the appropriate term to popularize the concept. Karlman Wasserman in his acclaimed famed classic 1964 paper described changes in respiratory exchange ratio to noninvasively determine the "*onset of anaerobic metabolism*" and coined this phenomena the "*anaerobic threshold*" (Wasserman & McIlroy, 1964). Soon later, with the estimable help of Beaver, Wasserman also described the nonlinear increase in ventilation in relation to the oxygen uptake during incremental exercise using rapid responding analyzers and reporting breath by breath respiratory data (Wasserman et al., 1973). Since then, the popularity of the LT and VT during incremental exercise testing increased dramatically and all the recognition went for Wasserman.

A controversial finding came with the publication of a paper by Hughes, Turner and Brooks (1982), where they observed that the LT and VT during incremental exercise could be manipulated independently of each other, challenging the validation of VT to estimate the standard arterial LT (Beaver et al., 1985). The findings of Hughes, Turner and Brooks (1982) were criticized by Wasserman (1983) via the letters to the editor section, what yielded a robust reply from Brooks (1983). Based mainly on the example of McArdle syndrome patients, where a VT can be identify with no BLC response, Brooks suggested that the ventilatory "*anaerobic threshold*" should be discarded. The interpretations (not the identification methods) of the "*anaerobic*" threshold changed rapidly during the 1980s. Although not exempt from criticism, findings of tracer kinetic experiments couple with daring investigators like George A. Brooks, challenged the "*immortalized*" theory of the "*anaerobic*" threshold. The "*anaerobic*" threshold and "*oxygen debt*" theories were based on the fact that lactate is produced in muscle due to the lack of oxygen and that lactate is produced during exercise as a dead-end waste metabolite product that can only be removed during recovery. The observations that the lactate is formed and utilized continuously under both aerobic and anaerobic conditions provoked the "*cell to cell and intracellular lactate shuttle*" theories (Brooks, 1986; Brooks, 2000; Brooks, 2009; Gladden, 2004; Robergs, Ghiasvand, & Parker, 2004; Robergs, 2011) and changed our viewpoint concerning the sense of the "*anaerobic*" threshold concept. Regardless of the controversy of how the threshold should be named and determined, and what was its physiological meaning, during that time (the 1980s) there was a robust consensus indicating that the first arterial BLC increment above resting BLC values during incremental exercise (the first LT or the aerobic LT during incremental exercise or the "*Owles' Point*" during constant exercise workload tests) was the actual LT gold standard to

determine aerobic capacity, predict endurance performance and prescribe training intensities (Beaver et al. 1985).

A more accepted view of the LT among contemporary exercise physiologists is encompassed by the concept of the maximal lactate steady state (MLSS). Close in time to the finding of the *"Owles' point"* (Owles, 1930), under the supervision of professors Krogh, Lindhard and Henriques, Ole Bang (1936) demonstrated that there was a second critical exercise intensity point during constant exercise workload tests, at a higher exercise intensity than the *"Owles' point"*, above which BLC progressively increased. By the measurements of more than 2 BLC data-points during constant exercise workloads, Bang (1936) showed that at constant exercise intensities close to the *"Owles' point"*, after the first rise in BLC during the initial 5-10 mins of exercise, BLC remained steady for 25-65 min. However, at higher exercise intensities, after the initial rise in BLC during the first 5-10 mins, a secondary increase in BLC could be observed. Therefore, the study of Bang (1936) suggested that there was a second critical exercise intensity level unique to each individual indicating the highest prolonged constant exercise intensity that after the initial rise in BLC does not produce further BLC increase. The intention to verify this idea drove exercise physiologist to the creation of the MLSS concept (Heck et al., 1985; LaFontaine, Londeree, & Spath, 1981; Londeree & Ames, 1975). The MLSS is encountered at higher exercise intensity than the first LT (≈130% relative to the first LT or the "*Owles' Point*") and is defined as the highest exercise workload showing no more than 1.0 mmol·L^{-1} increment from the 10th to the 30th min of constant workload exercise (Beneke, 1995). Nowadays the MLSS is considered the second gold standard for determination of aerobic capacity and training prescription in athletes. However, MLSS is not a real option for regular endurance testing. MLSS assessment is tedious, time consuming and demanding both for the athlete and the sport physiologist, as it requires 3 to 7 constant workload tests of about 30 min, full days of recovery between tests and diet restrictions to minimize effects on MLSS determination. Direct determination of MLSS is necessary if precision is required in experimental studies, but in real testing and monitoring of endurance capacity, such laborious aerobic diagnosis method is undesirable and unfeasible. Indeed, athletes and coaches commonly avoid such a laborious aerobic diagnosis method (Mann, Lamberts, & Lambert, 2013). Figure 5 shows on-field MLSS determination accomplished during one of our experimental studies (unpublished results) in an amateur soccer player between the end of March and mid of May 2016. Due to his training and competition schedule, no more than one constant velocity test per week could be performed. The figure clearly shows the tedious process of precisely (0.2 km·h^{-1} precision) determine the MLSS in competitive athletes. The logical refusal to this type of endurance diagnosis method that athletes and coaches usually express can be intuited from this figure. Hence, the MLSS has vigorously been attempted to estimate from a second critical exercise intensity point in the BLC-workload curve during a single incremental exercise test (example using data from the same soccer subject in Figure 6).

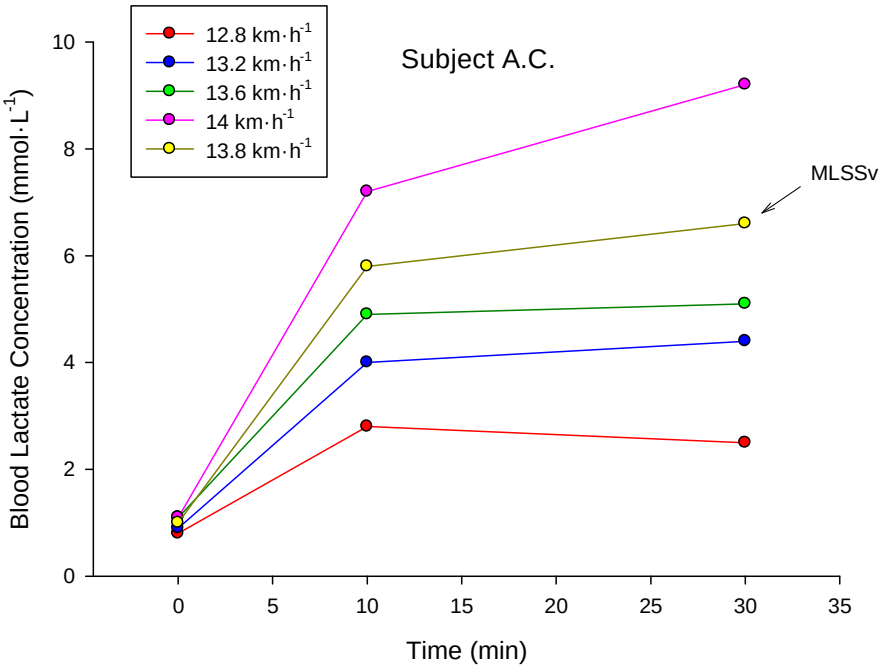

Figure 5: The maximal lactate steady state velocity (MLSSv) recently determined in a soccer player (A.C.). The highest velocity with an increase of no more than 1 mmol·L^{-1} from the 10th to the 30th min was the 13.8 km·h^{-1} velocity

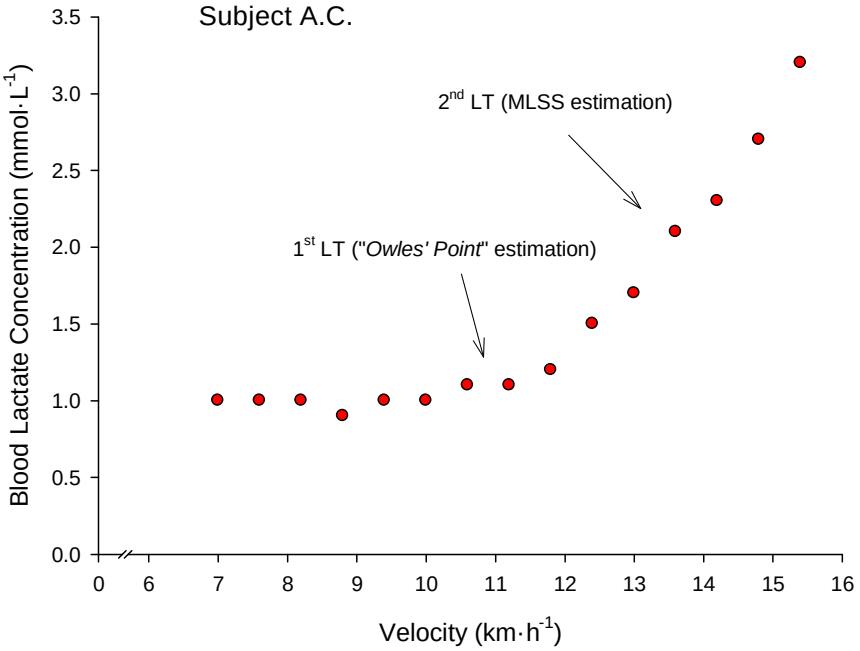

Figure 6: BLC-workload curve during incremental exercise in the same soccer player (A.C.) one week before the beginning of the MLSS determination showed in Figure 5. The 1st LT (*"Owles' Point"* estimation) occurred at about 10.6 km·h^{-1} and the 2nd LT (MLSS estimation) at 13.6 km·h^{-1}

This enthusiastic effort to estimate the MLSS from a single exercise test is clearly evidenced by the excessive methods described to detect and named this second threshold in the BLC-workload curve during incremental exercise, such as, the "*aerobic-anaerobic threshold*" (Kindermann, Simon, & Keul, 1979), "*minimum lactate equivalent*" (Berg, Stippig, Keul, & Huber, 1980; Roecker, Schotte, Niess, Horstmann, & Dickhuth, 1998), "*onset of blood lactate accumulation*" (Sjodin & Jacobs, 1981), "*individual anaerobic threshold*" (Stegmann, Kindermann, & Schnabel, 1981), "*Dmax method*" (Cheng et al., 1992), "*lactate turnpoint*" (Smith & Jones, 2001), etc. LT determination during a single incremental test as an estimation of the "*Owles' point"* and the MLSS is the most common method used among sport scientists to assess the physiological capacity of the subjects and prescribe endurance training in athletes, as well as in clinical special populations. According to Faude, Kindermann and Meyer (2009) nowadays there are more than 25 LT concepts. Figure 7 illustrates few of the possibilities to determine the LTs during incremental exercise testing. LT determination, thereby, requires qualified personnel not only for invasive blood sampling, but also for correct interpretation and appropriate handling of the results according to the needs of the athlete or the physical activity practitioner. The need of qualified professionals often hinders frequent (weekly or monthly) LT determination as would be required for ongoing monitoring of the aerobic capacity and proper adjustments in endurance training intensity, particularly in clinical special populations and athletes with limited resources. From my personal experience during my postgraduate period in different institutions (Studies, Research and Sports Medicine Centre of the Government of Navarre; Department of Health Sciences of the Public University of Navarre; Human Performance Laboratory of the University of Las Palmas de Gran Canarias; School of Life, Sport & Social Sciences of the Edinburgh Napier University; and the USP Araba Sport Clinic) I am quite confident by saying that LT testing is usually conducted 2-3 times per season in granted or financially supported teams and athletes, while the majority of the recreational club level athletes or clinical patients do not have the opportunity to determine their LTs so often. In these last kinds of subjects (athletes with limited resources or delicate individuals), alternative functional strategies to overcome actual on-field LT determination hitches are of great interest. The sports scientific community has therefore traditionally considered worthy to look for scientific methods requiring minimal equipment to assess aerobic capacity. There is a vast of literature provided by sport scientists such as Foster or Lucia demonstrating that heart rate (or heart rate relative to maximum heart rate) at the LTs remains quite steady over a competitive season in trained to elite athletes, suggesting that probably 2-3 laboratory or field LT assessments are enough to check that athletes and players are on track. However, the development of practical tools to estimate aerobic capacity in trained to elite athletes would allow continuous adjustments in the prescription of training intensity distribution, the tracking of the evolution of a deconditioned player during the final phase of an injury-rehabilitation or retraining process before returning to its previous conditioning level, or the evaluation of the level of fatigue generated by an overload session, period of training, or competition. Furthermore, the development of practical ways to estimate the LTs in athletes could point out the way to discover valuable tools to assess aerobic capacity in recreational athletes, and more importantly, in delicate clinical patients with limited resources.

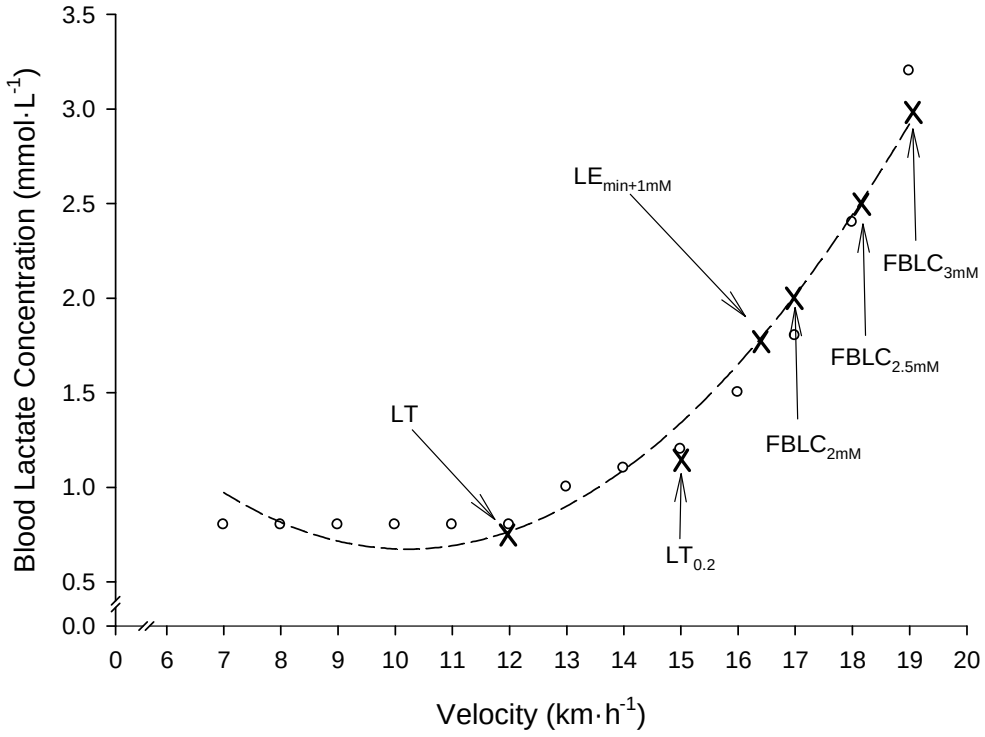

Figure 7: A typical polynomial blood lactate curve during incremental exercise obtained from an endurance trained runner (unpublished results) showing few of the possibilities to determine the 1st and 2nd lactate thresholds

In papers 2, 3 and 4 of this dissertation we aimed to develop scientific functional strategies requiring minimal equipment to indirectly determine the LTs by using data recorded in professional and elite athletes who had financial support for LT testing. We hope that our findings presented in these 3 papers helps athletes and coaches to be able to assess and monitor their endurance capacity more frequently, but we also hope that our findings spotlight the way to find out a real functional submaximal reliable test to be used in clinical delicate settings, and not just to spotlight a popular pastime for sport and exercise researches.

Aims and layouts of the thesis

Study 1

Chapter 2

Title: Gas analyzers' drift leads to systematic error in maximal oxygen uptake and maximal respiratory exchange ratio determination.

Research aim: To examine the drift in the measurements of fractional concentration of oxygen and carbon dioxide of a Nafion-using metabolic cart during incremental maximal exercise, and to propose a way in which the drift can be corrected.

Study 2

Chapter 3

Title: Heart rate-based prediction of fixed blood lactate thresholds in professional team-sport players.

Research aim: To investigate whether the speed associated with 90% of maximal heart rate could predict the widely used fixed blood lactate concentration thresholds in professional team-sport players.

Study 3

Chapter 4

Title: Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists.

Research aim: To predict the widely used aerobic and anaerobic lactate thresholds from mathematically determined heart rate variability thresholds in professional world-class road cyclists.

Study 4

Chapter 5

Title: Validity of a single lactate measure to predict fixed lactate thresholds in elite athletes

Research aim: To validate the use of a single blood lactate concentration measure taken following a 12 $km \cdot h^{-1}$ running stage to predict and monitor fixed blood lactate concentration thresholds in elite and professional athletes.

List of Abbreviations

Abbreviation	Meaning
AeT	Aerobic lactate threshold
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AnT	Anaerobic lactate threshold
BLC	Blood lactate concentration
BLC_{12}	Blood lactate concentration at 12 $km \cdot h^{-1}$
BMI	Body mass index
CI	Confidence interval
CO_2	Carbon dioxide
CV	Coefficient of variation
FBLC	Fixed blood lactate concentration
FCO_2	Fractional concentration of carbon dioxide
F_ECO_2	Expired fractional concentration of carbon dioxide
FeN_2	Expired fractional concentration of nitrogen
F_EO_2	Expired fractional concentration of oxygen
F_ICO_2	Inspired fractional concentration of carbon dioxide
F_IN_2	Inspired fractional concentration of nitrogen
F_IO_2	Inspired fractional concentration of oxygen
FO_2	Fractional concentration of oxygen
HF	Humidity factor
HR	Heart rate
HR_{max}	Maximal heart rate
HR_{peak}	Peak heart rate

HRV	Heart rate variability
HRVT	Heart rate variability threshold
$[La^-]$	Blood lactate concentration
$[La^-_{peak}]$	Peak blood lactate concentration
LT	Lactate threshold
MET	Metabolic equivalent
MLSS	Maximal lactate steady state
$MLSS_{int}$	Exercise intensity at the maximal lactate steady state
OBLA	Onset of blood lactate accumulation
O_2	Oxygen
PNS	Parasympathetic nervous system
P_B	Barometric pressure
PH_2O	Pressure of water vapor
r	Pearson's product-moment correlation coefficient
R^2	Coefficient of determination
RER	Respiratory exchange ratio
RER_{max}	Maximal respiratory exchange ratio
$_RH$	Room humidity
Rpm	Revolutions per minute
RR interval	R wave to R wave interval
$_RT$	Room temperature
SD	Standard deviation
SD1	standard deviation of the instantaneous beat-to-beat RR intervals
SD1T	SD1 threshold
SEE	Standard error of the estimate

SNS	Sympathetic nervous system
SPSS	Statistical Package for the Social Sciences
STPD	Standard conditions for temperature, pressure and dry
S3mM	Running speed at fixed blood lactate concentrations of 3 $mmol \cdot L^{-1}$
S4mM	Running speed at fixed blood lactate concentrations of 4 $mmol \cdot L^{-1}$
$S90\%HR_{max}$	Running speed at 90% of maximal heart rate
T1	Testing session before an intensified training period
T2	Testing session after an intensified training period
$\dot{V}co_2$	Carbon dioxide output
$\dot{V}co_{2max}$	Maximal carbon dioxide output
$\dot{V}_E$	Minute ventilation
$\dot{V}_{E_{max}}$	Maximal Minute ventilation
$\dot{V}o_2$	Oxygen uptake
$\dot{V}o_{2max}$	Maximal oxygen uptake
W_{peak}	Peak aerobic power

References

Astrand, P. O. & Saltin, B. (1961a). Maximal oxygen uptake and heart rate in various types of muscular activity. *J Appl.Physiol, 16,* 977-981.

Astrand, P. O. & Saltin, B. (1961b). Oxygen uptake during the first minutes of heavy muscular exercise. *J Appl.Physiol, 16,* 971-976.

Atkinson, G., Davison, R. C., & Nevill, A. M. (2005). Performance characteristics of gas analysis systems: what we know and what we need to know. *Int.J Sports Med., 26 Suppl 1,* S2-10.

Bang, O. (1936). The lactate content of the blood during and after muscular exercise in man. *Skandinavisches Archiv Für Physiologie*, *74*(S10), 51-82.

Bassett, D. R. Jr. (2002). Scientific contributions of A. V. Hill: exercise physiology pioneer. *J Appl.Physiol (1985.), 93,* 1567-1582.

Bassett, D. R. Jr. & Howley, E. T. (1997). Maximal oxygen uptake: "classical" versus "contemporary" viewpoints. *Med.Sci.Sports Exerc, 29,* 591-603.

Bassett, D. R. Jr. & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med.Sci.Sports Exerc, 32,* 70-84.

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1973). On-line computer analysis and breath-by-breath graphical display of exercise function tests. *J Appl.Physiol, 34,* 128-132.

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1985). Improved detection of lactate threshold during exercise using a log-log transformation. *J Appl.Physiol (1985.), 59,* 1936-1940.

Beneke, R. (1995). Anaerobic threshold, individual anaerobic threshold, and maximal lactate steady state in rowing. *Med Sci.Sports Exerc, 27,* 863-867.

Berg, A., Stippig, J., Keul, J., & Huber, G. (1980). Zur Beurteilung der Leistungsfähigkeit und Belastbarkeit von Patienten mit coronarer Herzkrankheit. *Dtsch Z Sportmed, 31,* 205.

Bergh, U., Ekblom, B., & Astrand, P. O. (2000). Maximal oxygen uptake "classical" versus "contemporary" viewpoints. *Med Sci.Sports Exerc, 32,* 85-88.

Brooks, G. A. (1983). Misconceptions and missed perceptions of the anaerobic threshold. *J Appl.Physiol Respir.Environ.Exerc Physiol, 54,* 853-854.

Brooks, G. A. (1986). The lactate shuttle during exercise and recovery. *Med Sci.Sports Exerc, 18,* 360-368.

Brooks, G. A. (2000). Intra- and extra-cellular lactate shuttles. *Med Sci.Sports Exerc, 32,* 790-799.

Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *J Physiol, 587,* 5591-5600.

Buchholz, A. C. & Schoeller, D. A. (2004). Is a calorie a calorie? *Am.J Clin.Nutr., 79,* 899S-906S.

Calbet, J. (2006). Fisiología del ejercicio: concepto y revisión histórica. In J.Chicharro & A. Vaquero (Eds.), *Fisiología del ejercicio* (pp. 1-32). Madrid: Médica Panamerica.

Cheng, B., Kuipers, H., Snyder, A. C., Keizer, H. A., Jeukendrup, A., & Hesselink, M. (1992). A new approach for the determination of ventilatory and lactate thresholds. *Int.J Sports Med, 13,* 518-522.

Coyle, E. F. (1995). Integration of the physiological factors determining endurance performance ability. *Exerc Sport Sci.Rev., 23,* 25-63.

Douglas, C. G. (1911). A method for determining the total respiratory exchange in man. *J Physiol,* 1-2.

Duveen, D. I., & Klickstein, H. S. (1955). Antoine Laurent Lavoisier's contributions to medicine and public health. *Bull.Hist Med., 29,* 164-179.

Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate threshold concepts. How valid are they? Sports Med, 23(6), 469-490

Gladden, L. B. (2004). Lactate metabolism: a new paradigm for the third millennium. *J Physiol, 558,* 5-30.

Gore, C. J., Tanner, R. K., Fuller, K. L., & Stanef, F. (2013). Determination of maximal oxygen consumption (VO2max). In R.K.Tanner & C. J. Gore (Eds.), *Physiological tests for elite athletes* (pp. 103-122). Champaign, IL: Human Kinetics.

Graham, T. E. (1984). Mechanisms of blood lactate increase during exercise. *Physiologist, 27,* 299-303.

Hale, T. (2008). History of developments in sport and exercise physiology: A. V. Hill, maximal oxygen uptake, and oxygen debt. *J Sports Sci., 26,* 365-400.

Hawkins, M. N., Raven, P. B., Snell, P. G., Stray-Gundersen, J., & Levine, B. D. (2007). Maximal oxygen uptake as a parametric measure of cardiorespiratory capacity. *Med.Sci.Sports Exerc, 39,* 103-107.

Heck, H., Mader, A., Hess, G., Mucke, S., Muller, R., & Hollmann, W. (1985). Justification of the 4-mmol/l lactate threshold. *Int.J Sports Med, 6,* 117-130.

Hill, A., Long, C., & Lupton, H. (1924a). Musucular exercise, lactic acid, and the supply and utilization of oxygen-Parts IV-VI. *Proc Roy Soc B, 97,* 84-138.

Hill, A., Long, C., & Lupton, H. (1924b). Musucular exercise, lactic acid, and the supply and utilization of oxygen-Parts VII-VIII. *Proc Roy Soc B, 97,* 155-176.

Hill, A. & Lupton, H. (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Q J Med, 16,* 135-171.

Hollmann, W. (1985). Historical remarks on the development of the aerobic-anaerobic threshold up to 1966. *Int.J Sports Med, 6,* 109-116.

Hollmann, W. (2001). 42 years ago--development of the concepts of ventilatory and lactate threshold. *Sports Med, 31,* 315-320.

Howley, E. T., Bassett, D. R. Jr., & Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Med.Sci.Sports Exerc., 27,* 1292-1301.

Hughes, E. F., Turner, S. C., & Brooks, G. A. (1982). Effects of glycogen depletion and pedaling speed on "anaerobic threshold". *J Appl.Physiol Respir.Environ.Exerc Physiol, 52,* 1598-1607.

Jones, N. L. & Ehrsam, R. E. (1982). The anaerobic threshold. *Exerc Sport Sci.Rev., 10,* 49-83.

Joyner, M. J. (1991). Modeling: optimal marathon performance on the basis of physiological factors. *J Appl.Physiol (1985.), 70,* 683-687.

Joyner, M. J. & Coyle, E. F. (2008). Endurance exercise performance: the physiology of champions. *J Physiol, 586,* 35-44.

Kindermann, W., Simon, G., & Keul, J. (1979). The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur.J Appl.Physiol Occup.Physiol, 42,* 25-34.

Kissen, A. T. & McGuire, D. W. (1967). New approach for on-line, continuous determination of oxygen consumption in human subjects. *Aerosp.Med, 38,* 686-689.

LaFontaine, T. P., Londeree, B. R., & Spath, W. K. (1981). The maximal steady state versus selected running events. *Med Sci.Sports Exerc, 13,* 190-193.

Leger, L. & Mercier, D. (1984). Gross energy cost of horizontal treadmill and track running. *Sports Med, 1,* 270-277.

Levine, B. D. (2008). .VO2max: what do we know, and what do we still need to know? *J Physiol, 586,* 25-34.

Londeree, B. R. & Ames, S. A. (1975). Maximal steady state versus state of conditioning. *Eur.J Appl.Physiol Occup.Physiol, 34,* 269-278.

Lundby, C. & Robach, P. (2015). Performance Enhancement: What Are the Physiological Limits? *Physiology.(Bethesda.), 30,* 282-292.

Mann, T., Lamberts, R. P., & Lambert, M. I. (2013). Methods of prescribing relative exercise intensity: physiological and practical considerations. *Sports Med, 43,* 613-625.

McArdle, W., Katch, F., & Katch, V. (2001). *Exercise physiology. Energy nutrition and human performance*. (5 ed.) Baltimore: Limppincott Williams & Wilkins.

Mitchell, J. H., Sproule, B. J., & Chapman, C. B. (1958). The physiological meaning of the maximal oxygen intake test. *J Clin.Invest, 37,* 538-547.

Noakes, T. D. (1988). Implications of exercise testing for prediction of athletic performance: a contemporary perspective. *Med.Sci.Sports Exerc, 20,* 319-330.

Noakes, T. D. (1997). 1996 J.B. Wolffe Memorial Lecture. Challenging beliefs: ex Africa semper aliquid novi. *Med.Sci.Sports Exerc, 29,* 571-590.

Noakes, T. D. (1998). Maximal oxygen uptake: "classical" versus "contemporary" viewpoints: a rebuttal. *Med.Sci.Sports Exerc, 30,* 1381-1398.

Noakes, T. D. & Marino, F. E. (2009). Point: maximal oxygen uptake is limited by a central nervous system governor. *J Appl.Physiol (1985.), 106,* 338-339.

Noakes, T. D., Peltonen, J. E., & Rusko, H. K. (2001). Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *J Exp.Biol., 204,* 3225-3234.

Owles, W. H. (1930). Alterations in the lactic acid content of the blood as a result of light exercise, and associated changes in the co(2)-combining power of the blood and in the alveolar co(2) pressure. *J Physiol, 69,* 214-237.

Robergs, R. A. (2011). Nothing 'evil' and no 'conundrum' about muscle lactate production. *Exp.Physiol, 96,* 1097-1098.

Robergs, R. A., Ghiasvand, F., & Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *Am.J Physiol Regul.Integr.Comp Physiol, 287,* R502-R516.

Robergs, R. (2001). An exercise physiologist's "contemporary" interpretations of the "ugly and creaking edifices" of the VO2max concept. *J Exerc Physiol Online, 4,* 1-44.

Roecker, K., Schotte, O., Niess, A. M., Horstmann, T., & Dickhuth, H. H. (1998). Predicting competition performance in long-distance running by means of a treadmill test. *Med Sci.Sports Exerc, 30,* 1552-1557.

Saltin, B. & Astrand, P. O. (1967). Maximal oxygen uptake in athletes. *J Appl.Physiol, 23,* 353-358.

Sjodin, B. & Jacobs, I. (1981). Onset of blood lactate accumulation and marathon running performance. *Int.J Sports Med, 2,* 23-26.

Smith, C. G. & Jones, A. M. (2001). The relationship between critical velocity, maximal lactate steady-state velocity and lactate turnpoint velocity in runners. *Eur.J Appl.Physiol, 85,* 19-26.

Stegmann, H., Kindermann, W., & Schnabel, A. (1981). Lactate kinetics and individual anaerobic threshold. *Int.J Sports Med, 2,* 160-165.

Taylor, H. L., Buskirk, E., & Henschel, A. (1955). Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl.Physiol, 8,* 73-80.

Wasserman, K. (1983). Misconceptions and missed perceptions of the anaerobic threshold. *J Appl.Physiol Respir.Environ.Exerc Physiol, 54,* 853-854.

Wasserman, K. & McIlroy, M. B. (1964). Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *Am.J Cardiol., 14,* 844-852.

Wasserman, K., Whipp, B. J., Koyl, S. N., & Beaver, W. L. (1973). Anaerobic threshold and respiratory gas exchange during exercise. *J Appl.Physiol, 35,* 236-243.

Weltman A (1995). *The blood lactate response to exercise*. Human Kinetics, Champaign

Whipp, B. J., Davis, J. A., Torres, F., & Wasserman, K. (1981). A test to determine parameters of aerobic function during exercise. *J Appl.Physiol Respir.Environ.Exerc Physiol, 50,* 217-221.

Winter, E. M. (2012). Calibration and verification of instruments. *J Sports Sci., 30,* 1197-1198.

Chapter 2

Gas analyzers' drift leads to systematic error in maximal oxygen uptake and maximal respiratory exchange ratio determination

* Garcia-Tabar I, Eclache JP, Aramendi JF, Gorostiaga EM. Gas analyzers' drift leads to systematic error in maximal oxygen uptake and maximal respiratory exchange ratio determination. *Frontiers in Physiology* 2015; 30(6):308.

Gas analyzer's drift leads to systematic error in maximal oxygen uptake and maximal respiratory exchange ratio determination

Authors

Ibai Garcia-Tabar[1], Jean P. Eclache[2], José F. Aramendi[1] and Esteban M. Gorostiaga[1]

Affiliations

[1]Studies, Research and Sports Medicine Center, Government of Navarre, Pamplona, Spain

[2]Laboratory of Performance, Sport-Occupational Activities-Biology-Association, Lyon-Chassieu, France

Abstract

The aim was to examine the drift in the measurements of fractional concentration of oxygen (FO_2) and carbon dioxide (FCO_2) of a Nafion-using metabolic cart during incremental maximal exercise in 18 young and 12 elderly males, and to propose a way in which the drift can be corrected. The drift was verified by comparing the pre-test calibration values with the immediate post-test verification values of the calibration gases. The system demonstrated an average downscale drift (P<0.001) in FO_2 and FCO_2 of -0.18% and -0.05%, respectively. Compared with measured values, corrected average maximal oxygen uptake values were 5-6% lower (P<0.001) whereas corrected maximal respiratory exchange ratio values were 8-9% higher (P<0.001). The drift was not due to an electronic instability in the analyzers because it was reverted after 20 minutes of recovery from the end of the exercise. The drift may be related to an incomplete removal of water vapor from the expired gas during transit through the Nafion conducting tube. These data demonstrate the importance of checking FO_2 and FCO_2 values by regular pre-test calibrations and post-test verifications, and also the importance of correcting a possible shift immediately after exercise.

***Keywords*:** exercise testing, maximal oxygen consumption, gas exchange, calibration, verification

Introduction

Maximal oxygen uptake ($\dot{V}o_{2max}$) is defined as the highest rate at which oxygen can be taken up and utilized by the body during exercise. In laboratory settings, $\dot{V}o_{2max}$is commonly measured during incremental exercise to exhaustion, during which expired air is analyzed. The key variables needed to calculate $\dot{V}o_{2max}$ are the ventilator flow and the inspired and the expired fractional concentrations of oxygen (F_IO_2 and F_EO_2, respectively) and carbon dioxide (F_ICO_2 and F_ECO_2, respectively) (Gore, Tanner, Fuller, & Stanef, 2013; Hodges, Brodie, & Bromley, 2005).

One of the main potential sources of error in the calculation of $\dot{V}o_{2max}$ using automated systems is related to the stability of F_EO_2 and F_ECO_2 measurements, because the electronic oxygen (O_2) and carbon dioxide (CO_2) analyzers are prone to drift over time (Gore et al., 2013; Winter, 2012). To our knowledge, there is surprisingly relatively little information available on the stability of O_2 and CO_2analyzing systems over time during incremental exercise (Hodges et al., 2005; Salier, Rosdahl, & Schantz, 2012). In virtually all the publications that have measured$\dot{V}o_{2max}$, the authors have mentioned performing a pre-test calibration. As it has been pointed out in a recent Editorial (Winter, 2012)**,** in the majority of these studies it is rare to see, however, equivalent post-test verifications. For instance, after reviewing more than 50 studies measuring $\dot{V}o_{2max}$published between 1973 and 2012, we have found only 8 studies (≈16%) in which the authors mentioned that the analyzers' drift at the completion of exercise was assessed (Armstrong & Costill, 1985; Day, Rossiter, Coats, Skasick, & Whipp, 2003; Gore, Clark, Shipp, van der Ploeg, & Withers, 2003; McLaughlin, King, Howley, Bassett, Jr., & Ainsworth, 2001; Prieur et al., 1998; Rietjens, Kuipers, Kester, & Keizer, 2001; Wilmore, Davis, & Norton, 1976; Bowen et al., 2012). Only 4 of these 8 studies reported the average numerical drift values in O_2% and CO_2% (Armstrong & Costill, 1985; Prieur et al., 1998; Rietjens et al., 2001; Wilmore et al., 1976), which ranged from 0.02% to 0.22%. These reported drift values, according to the equations governing gas concentrations (Beaver, Wasserman, & Whipp, 1973; Wasserman, Hansen, Sue, Whipp, & Casaburi, 1994), would have caused an error in $\dot{V}o_{2max}$ up to 8-9% in standard laboratory conditions (~20ºC of temperature, ~40% of relative humidity and ~720 mmHg of barometric pressure). Furthermore, none of these 4 studies gave any criterion for the maximum drift error that can be accepted. It is still unknown whether the drift magnitude is related to some physical or physiological exercise variables and how long any particular drift remains after the end of exercise. It is also unclear how the drift readings should be adjusted or corrected to overcome the inaccuracy due to the drift (Winter, 2012).

Clearly, it seems that the process of post-test verification tends to be overlooked and there is insufficient data available on how stable specific gas analysis systems are during exercise conditions (Atkinson, Davison, & Nevill, 2005; Salier et al., 2012). This issue may be particularly relevant in several modern analyzers, in which the exhaled gas is not dried but is equilibrated with the laboratory environment by the use of a length of semi-permeable Nafion tubing (Larsson, Wadell, Jakobsson, Burlin, & Henriksson-Larsen, 2004; Medbø, Mamen, Welde, von Heimburg E., & Stokke, 2002). The purpose of the present study was, therefore, to examine the drift over time of a Nafion-using O_2 and CO_2analyzing system during maximal incremental exercise in experienced athletes and elderly sedentary males. By including sedentary elderly and young athletic subjects, as well as short and long-duration exercise protocols, a large range of metabolic

responses and exercise durations were examined and the influence of the drift on oxygen uptake ($\dot{V}o_2$), CO_2 output ($\dot{V}co_2$) and respiratory exchange ratio (RER) assessment was determined. This study also proposed a way in which the error might be reduced.

Materials and Methods

Subjects

Eighteen male amateur athletes (young group) and twelve older men (elderly group) volunteered to participate in the study. Athletes were recruited from various regional Sports Federations (Swimming, Athletics, Basketball, Basque-Ball, Paddle Tennis, Mountaineering and Climbing, Karate, Taekwondo, Judo and Boxing). Athletes' mean (± SD) age, height, body mass and percentage of body fat were 22 ± 6 years, 182 ± 7 cm, 79.3 ± 8.3 kg and 10.4 ± 3.1%, respectively. Participants in the elderly group were recruited from a Physical Activity Program for persons over 55. Mean (± SD) age, height, body mass and percentage of body fat of the participants constituting the elderly group were 69 ± 6 years, 167 ± 7 cm, 85.9 ± 13.3 kg and 27.3 ± 4.3%, respectively. A detailed medical history was taken on the day of the study. No subject reported a history of abnormal dyspnea on exertion or of angina.

Written informed consent was obtained from all volunteers prior to their participation. The study was approved by the Institutional Review Committee of the Instituto Navarro del Deporte y Jueventud (Government of Navarre, Spain), according to the requirements of the Declaration of Helsinki.

Exercise trials

Two different maximal incremental exercise protocols, with different exercise stage duration, were used for each population to examine whether the drift is influenced by the duration of the test. All testing sessions within each group were performed at the same time of the day in an air-controlled and well ventilated laboratory with a volume of1,121 m^3. Young and elderly individuals reported to the laboratory at least 2 h after their last meal and having refrained from caffeine, alcohol, and strenuous or non-habitual exercise for 24 h before testing.

Young exercise trials

Participants were habituated to the exercise testing equipment and procedures, as they were previously tested in the same laboratory using similar testing procedures. $\dot{V}o_{2max}$ was determined by a continuous maximal graded exercise test while sitting on a mechanically braked cycle-ergometer (Monark, Ergomedic 839-E, Varberg, Sweden). The exercise started at 20 W and the load was increased by 20 W every 2 min until volitional exhaustion. This exercise protocol was designed to reach volitional exhaustion within 23 to 33 min. It has been shown that relatively short (8-12 min) or long (~ 30 min) protocols do not affect attainment of $\dot{V}o_{2max}$ in highly motivated athletes (Gore et al., 2013). Participants maintained a constant cycling pedaling cadence of 60 rpm. Exhaustion was defined as the subject not being able to maintain the required pedaling cadence, despite vigorous verbal encouragement during the last min of exercise.

Elderly exercise trials

$\dot{V}o_{2max}$ was determined by a continuous incremental maximal exercise test on a treadmill ergometer (Kuntaväline, Hyper Treadmill 2040, Finland). The exercise test started at 5.5 $km{\cdot}h^{-1}$, after one min the speed was increased to 6.1 $km{\cdot}h^{-1}$for another min, and thereafter grade was

increased 1.1% every min until volitional exertion. Exhaustion was defined as the subjects not being able to maintain the required exercise intensity or they wished to stop.

At least two of the following criteria had to be met to determine $\dot{V}o_{2max}$ in both groups (American College of Sports Medicine, 2009): 1) no increase in $\dot{V}o_2$despite increased workload, defined as a $\dot{V}o_2$ increment of less than 120 ml·min^{-1} per stage in the young group or a $\dot{V}o_2$ increment of less than 1.75 ml·kg^{-1}·min^{-1} per stage in the elderly group. This criterion implies that any increment lower than 50% of the metabolic demand of these protocols' stages was accepted as a $\dot{V}o_2$ plateau (Taylor, Buskirk, & Henschel, 1955). 2) A maximal respiratory exchange ratio (RER_{max}) greater than 1.10 (Robergs, Dwyer, & Astorino, 2010); 3) peak blood lactate concentration greater than 8 mmol·L^{-1}, and 4) peak heart rate exceeding 90% of age predicted maximum (220-age). Heart rate (Polar Electro Oy, RS800CX, Kempele, Finland) was monitored throughout the exercise in both groups. Capillary blood samples from hyperemic earlobe were obtained at rest, on completion of the trial and at the 1st and 3rd min of recovery. After cleaning and puncturing, the single-use enzyme-coated electrode test strip was directly filled by a 5 *µl* whole-blood sample and blood lactate concentration was amperometrically determined (Arkray KDK Corporation, Lactate Pro LT-1710, Shiga, Japan).

Collection of respiratory gases

Participants were fitted with an appropriately sized mouth and nasal breathing mask (Series 7930, Hans Rudolph, Kansas City, MO, USA) adjusted with a headgear (Vacu-Med, Ventura, CA, USA). Metabolic data was continuously collected using a Vista Mini-CPX (Vacu-Med, Silver Edition 17670, Ventura, CA, USA) computer-integrated metabolic system. The Vista Mini-CPX is a high precision mass flowmeter instrument composed of a turbine flow sensor and O_2 and CO_2 analyzers designed to measure the flow of the exhaled gases and the concentrations in the O_2 and CO_2 gases on-line. At the start of each test, room temperature ($_RT$), barometric pressure (P_B), and relative room humidity ($_RH$) were measured (Precision Barometer, Lufft, Fellbach, Germany) and these data were entered manually into the computer. The environmental laboratory conditions were kept within the recommended values (18 to 23ºC with a relative humidity lower than 70%) (Gore et al., 2013)by means of a heating system.

Minute expired ventilation ($\dot{V}_E$) is calculated by a signal generated by the volume transducer of the turbine flow sensor. F_EO_2 is measured at $_RH$ through a disposable galvanic fuel cell (Teledyne Analytical Instruments, R-22MED Oxygen Sensor, Industry, CA, USA). F_ECO_2 is measured at $_RH$ through a nondispersive infrared system (Servomex, Ir1507 CO_2 infrared transducer, Crowborough, UK). According to the manufacturer, the CO_2 and O_2 analyzers have zero drift (< 1.5 Torr in 1 h for the CO_2 analyzer and 0.3% a week at constant temperature for the O_2 analyzer) and their response times are 90 to 130 ms (CO_2 analyzer) and 5 sec (O_2 analyzer). This time delay is automatically assessed and the length of the airline is taken into account according to the manufacturer's specifications. From these measurements the metabolic cart's computer calculates the mass flow of $\dot{V}o_2$ (in liters per minute), $\dot{V}co_2$ (in liters per minute), and the ratio of $\dot{V}o_2$ to $\dot{V}co_2$ (RER) with an accuracy (according to the manufacturer) of ± 1% in measures of F_EO_2 and F_ECO_2, of ± 2% in measures of $\dot{V}_E$, and of ± 3% in measures of $\dot{V}o_2$ and $\dot{V}co_2$.

This metabolic system uses a proportional sampling approach in the process of mixing the exhaled gases. Thus, the flow rate of this sampling is closely related to the flow of exhalation at ~ 0.5% of its rate, and directs the exhaled gases in three steps into the O_2 and CO_2 gas analyzers connected in parallel: 1) through a capillary tube, into a miniature mixing chamber, 2) through a built-in Nafion gas dryer humidifier conducting 180 tube (29 cm long x 1 mm inner diameter), and 3) through a capillary tube system with the same configuration of sampling tube length, diameter and pump flow rate for both analyzers. The Nafion tube is a semi-permeable membrane to water vapor made of copolymer of tetrafluoroethylene (Teflon®) and perfluoro-3,6-dioxa-4-methyl-7-octene-sulfonic acid, highly selective in the removal of water from the vapor phase. The Nafion tube allows water vapor to pass in and out of the tube by absorption and conveys the exhaled gases to the gas analyzers once an equilibrium is reached with the ambient humidity (Macfarlane, 2001). According to the Nafion manufacturer, during exercise the water vapor tension of the aspirated gas sample (relative humidity ~ 100%) (Atkinson et al., 2005; Bageant, 1976; Macfarlane, 2001) is reduced in milliseconds to the level of $_R$H of the laboratory environment (~27%, **Table 1**) by moving the water through the Nafion membrane wall and evaporating it very quickly into the surrounding air. Conversely, the typically dry calibration gas is humidified by the Nafion tubing to the level of $_R$H. This system provides a constant value of water vapor tension of the exhaled and calibration gases just prior to the entry of the samples into the gas analyzers. The Nafion tube was replaced at least every 3 years according to the manufacturer. All tests were carried out within the 18 months following the last Nafion tube replacement.

Table 1 Room environmental conditions (mean ± SD) during the exercise and non-exercise trials

	Young Exercise Trials (n = 18)	Young Non-exercise Trials (n = 18)	Elderly Exercise Trials (n = 12)	Elderly Non-Exercise Trials (n = 12)
Temperature (°C)	21.0 ± 1.2	20.4 ± 0.5	20.4 ± 0.5	20.1 ± 0.3
Humidity (%)	27 ± 6	28 ± 4	28 ± 4	24 ± 1
Pressure (mmHG)	726 ± 5*	716 ± 4	716 ± 4	715 ± 4

* Significantly different from the simulated trials; *P*< 0.01

The metabolic measurement software supplied with the analyzer (Vacu-Med, TurboFit 5, Ventura, CA, USA) was set to report mean metabolic data over a 30 sec time period and to adjust the volume of the expired air to standard conditions (STPD) for temperature (0ºC), pressure (760 mmHg) and dry (absence of water vapor). $\dot{V}o_{2max}$ was defined as the highest 30-sec $\dot{V}o_2$ value averaged over two consecutive readings, and its time-corresponding values of $\dot{V}co_2$, $\dot{V}_E$ and RER were considered as $\dot{V}co_{2max}$, $\dot{V}_{E_{max}}$ and RER_{max}, respectively.

Pre-test calibration and post-test verification processes

The instrument was warmed up for at least 2 h prior to every exercise test to minimize any possible electrical drift. Calibration of the O_2 and CO_2 analyzers was performed immediately prior to every test using two-point calibration with two precision-analyzed gas mixtures. One calibration point was room air (O_2: 20.93%; CO_2: 0.00%) Non-hygroscopic soda lime CO_2

absorbent (Vacu-Med, Ventura, CA, USA) was used for maximum precision of ambient CO_2 measurement. Thus, fractional concentrations of room air were assumed to be 20.93% O_2 and 0.00% CO_2. The second point was a high-precision certified calibration tank gas containing 15.05% O_2, 5.99% CO_2 and balanced nitrogen. This high-precision gas was determined gravimetrically, was obtained from a reliable gas supplier (Praxair, Madrid, Spain) and had a claimed accuracy of ± 0.02%. Turbine flow calibration was determined using a high-precision 3-L calibration syringe (Vacu-Med, Calibringe 1092, Ventura, CA, USA), in a five-pump series. A series of complete pumps of the syringe and of gas calibrations were repeated until the difference between the current and the previous calibration was less than 0.05 L for volume and less than 0.02% for O_2 and CO_2. When the calibration process was finished, the gas sample line was connected to the subject's mask.

Within 15 sec of the completion of each exercise trial the sample line was removed from the connection to the face mask/turbine and the after trial verification of FO_2, FCO_2 and turbine flow measurements was performed. Both calibration gases (room air and tank gas) were run through the metabolic system to check for the drift of the analyzer over the course of the measurement period. Verification readings of the calibration gases and the flow sensor were noted down and compared with the calibration references.

Correction of metabolic data

Post-test verifications readings were used to correct the metabolic data measured by the Vista Mini-CPX. Corrected $\dot{V}_E$ ($\dot{V}_{E_c}$) in STPD condition was calculated as follows:

$$\dot{V}_{E_c} = 3 \cdot \dot{V}_{E_{me}} \cdot [\text{Cal} + (\text{Ver} - \text{Cal})]^{-1}$$

$$\dot{V}_{E_c} = 3 \cdot \dot{V}_{E_{me}} \cdot [\text{Ver}]^{-1}$$

where "3" was the volume (L) of the syringe used to calibrate the flow sensor, "$\dot{V}_{E_{me}}$" was the minute ventilation ($L \cdot min^{-1}$) in STPD condition measured by the metabolic cart, "Cal" was the calibration readout (L) recorded before the exercise and "Ver" was the verification readout (L) recorded after the exercise.

Correction of FO_2 is illustrated in **Figure 1**. During the pre-test calibration process we adjusted the gain settings of the span potentiometers to the corresponding voltage outputs, so that readings of O_2% (tank gas: y_1 = 15.05%; room air: y_2 = 20.93%) equaled real O_2% ($x_1 = y_1$; $x_2 = y_2$). The equation of the pre-test calibration regression line is therefore:

$$Y = X$$

During the post-exercise verification process we used the same pre-test calibration gases (x_1 = 15.05% O_2; x_2 = 20.93% O_2), but the %O_2 values read (y'_1 and y'_2) were different from the O_2% read during the pre-test calibration process. In this case, the equation of the post-test verification regression line is:

$$Y = A' \cdot X + B'$$

Being:

$$A' = (y'_2 - y'_1) / (x_2 - x_1)$$

$$B' = y'_2 - (A' \cdot x_2)$$

For a given value of (y'_n) measured at $\dot{V}o_{2max}$ during exercise, we can calculate the corresponding value of x (x_n) from the equation of the post-test verification line (Y = A'X + B') as follows:

$$y'_n = A' \cdot x_n + B'$$

$$x_n = (y'_n - B') / A'$$

Therefore, the corrected F_EO_2 value at $\dot{V}o_{2max}$ (y'_n) in the pre-test calibration line (Y = X) is:

$$y'_n = x_n$$

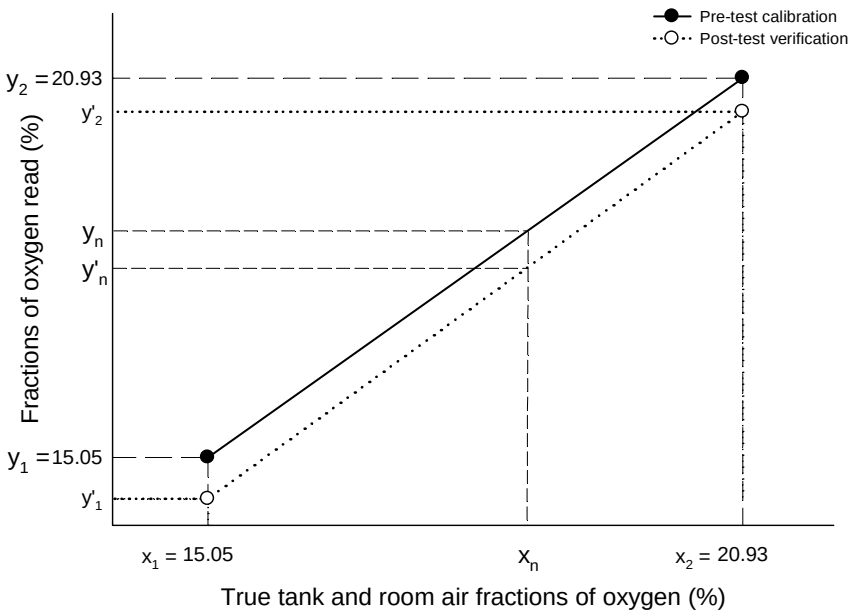

Figure 1: Correction of fractional concentrations of oxygen. x_1 and x_2,true tank (x_1 = 15.05%) and room air (x_2= 20.93%) fractions of oxygen; y_1 and y_2, fractions of tank (y_1 = 15.05%) and room oxygen (y_2= 20.93%) read by the analyzer during the pre-test calibration process when the true tank (x_1) and room air (x_2) gases were aspirated by the analyzers; y'_1 and y'_2, fractions of tank (y'_1) and room oxygen (y'_2) read by the oxygen analyzer during the post-test verification process when true tank (x_1) and room air (x_2) gases were aspirated by the analyzers

F_ECO_2 was corrected using this same procedure. Once the corrected F_EO_2, F_ECO_2 and $\dot{V}_{E_c}$ were obtained, formulas provided by the manufacturer [see Beaver et al. (1973) or Wasserman et al. (1994) for further detail] were employed to correct$\dot{V}o_2$ and $\dot{V}co_2$ as follows:

$$\dot{V}co_2 = (F_ECO_2 - FiCO_2) \cdot \dot{V}_{E_c} \cdot HF$$

$$\dot{V}o_2 = [FiO_2 \cdot FeN_2 \cdot (FiN_2)^{-1} - F_EO_2] \cdot \dot{V}_{E_c} \cdot HF$$

where $FiCO_2$,FiO_2 and FiN_2 are fractions of inspired carbon dioxide, oxygen and nitrogen respectively, F_ECO_2,F_EO_2 and FeN_2 are fractions of corrected expired carbon dioxide, oxygen and nitrogen respectively, $\dot{V}_{E_c}$ is the corrected minute ventilation ($L \cdot min^{-1}$) in STPD condition, and HF is the humidity factor defined as:

$$HF = P_B - PH_2O \text{ (at } {}_RT, {}_RH) \cdot (P_B)^{-1}$$

where P_B is the barometric pressure (mmHg) and PH_2O is the pressure of water (mmHg) at room temperature ($_RT$) and humidity ($_RH$). Standard tables provided by the manufacturer, also presented by Wasserman et al. (1994), were used to determine PH_2O.

The metabolic system calculates FiN_2 and FeN_2 using the next two formulas:

$$FiN_2 = 0.79 \cdot HF$$

$$FeN_2 = HF - F_EO_2 - F_ECO_2$$

where it is assumed that FiN_2 is constant and FeN_2 is the remaining fractional gas of HF, F_EO_2 and F_ECO_2.

All corrections were performed off-line using specific routines developed in a commercial software package (The MathWorks Inc., MATLAB R2008a, Natick, MA, USA).

Non-exercise trials

To check the stability of the analyzers, each exercise test was pair-matched on duration, time of the day and number of pre-test calibrations and post-test verifications assessed, with a non-exercise trial, accounting for a total of 30 non-exercise trials (one per subject). These non-exercise trials consisted of performing the identical calibration and verification processes of the gas analyzers over the same time interval to that used during each exercise trial. Between the calibrations and verifications, the metabolic system worked throughout but no subject was connected to the metabolic cart. No flow or volume measures were recorded.

Recovery trials

The pattern of change in FO_2 and FCO_2 during the first 30 min of recovery after the completion of the exercise trials, and after disconnecting the gas sample line from the mask, was investigated immediately after 9 exercise trials. These recovery trials consisted of performing the post-test verifications of the gas analyzers within 15 sec of the completion of each exercise trial, but also at 3, 5, 10, 15, 20 and 30 min of recovery from each exercise trial.

Statistics

Standard statistical methods were used for the calculation of means, standard deviations (SD), standard errors of the estimates (SEE) and confidence intervals (CI). Data were analyzed using parametric statistics following confirmation of normality, homoscedasticity, and when appropriate sphericity. Gas measure readings after the trials (verification readings) were compared with the concentrations of the standard calibration gases (calibration readings) using two-tailed one-sample Student's *t*-tests. Two-tailed Student's paired *t*-tests were used to analyze

differences between verification readings of the exercise trials and their paired non-exercise trials, as well as between the non-corrected (measured) and corrected values of the respiratory parameters. Respiratory values of the elderly and young groups were compared by two-tailed independent samples *t*-tests, with Levene's tests used to assess equality of variances. Relationships between variables of interest were assessed by linear regression analyses. Pearson product-moment correlation coefficients (r) were used to indicate the magnitude and direction of each linear relationship. The slopes of the regression lines in elderly and young groups were compared using analysis of covariance (ANCOVA). Differences between pre- and post-test values in FO_2 and FCO_2 during the recovery period were analyzed using one factor ANOVA with repeated measures. When significance was found, Student's *t*-test with Bonferroni correction for multiple comparisons was used to locate the significance. Significance was set at $P<0.05$. Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, USA). Data in the text, tables and figures are reported as mean ± SD.

Results

Exercise trials

As designed, the duration of the young cycling exercise trials (26:53 ± 3 min) was higher (P < 0.001) than the duration of the elderly treadmill exercise trials (9:29 ± 3 min). Maximal power output reached by young athletes was 294 ± 34 W (3.74 ± 0.54 W·kg^{-1}). Maximal grade attained by elderly individuals at 6.1 km·h^{-1} was 8.5 ± 3.9%. Young athletes attained significantly higher (P < 0.001) peak heart rate and peak blood lactate concentration values (195 ± 11 b·min^{-1} and 10.3 ± 2.2 mmol·L^{-1}) compared to elderly individuals (144 ± 24 b·min^{-1} and 6.6 ± 1.7 mmol·L^{-1}, respectively).

The pre-test calibration and post-test verification values of FO_2 and FCO_2 of the room air and tank gases assessed within 15 sec of the completion of each exercise trial in the whole group of subjects are presented in **Table 2**. The system showed a downscale drift (P < 0.001) in FO_2 and FCO_2 from pre- to post-test values in the exercise trials. Mean absolute differences between pre- and post-test values were -0.18% (room air) and –0.14% (tank gas) in O_2 and 0.00% (room air) and –0.05% (tank gas) in CO_2. Expressed as a percentage of the average pre-test calibration values, the magnitude of the downscale drift was similar (~ 0.9%) in both analyzers. There was no statistical difference (P = 0.08; 95% CI: -0.00 to 0.01 L) in the registered air volumes between post-test verification (2.99 ± 0.01 L) and pre-test calibration values (2.99 ± 0.01 L). This means that the calibration factor for ventilation volume was essentially constant throughout the test period.

Figure 2 presents the relationships in the total sample between the individual values of $\dot{V}_{E_{max}}$ and the individual post-test verification values of FO_2 and FCO_2 of both calibration gases (room air and tank gas). Regression analyses indicated significant negative correlations between $\dot{V}_{E_{max}}$ and post-test verification values of room air FO_2 in the total sample (r = - 0.48; P = 0.007; SEE = 0.056%; 95% CI: 20.77 to 20.91%) and in the young group (r = - 0.49; P = 0.03; SEE = 0.057%; 95% CI: 20.75 to 21.04%). The gradients of the rest of the relationships presented in **Figure 2** were not different from zero (P < 0.05). According to the ANCOVA results, the slopes of the regression lines were not different among groups (P > 0.05). No significant relationships were observed between test duration and post-test verification values of FO_2 and FCO_2 (P > 0.05).No other relevant significance was found between respiratory parameters and post-trial readings.

Non-exercise trials

During the non-exercise trials, the drift over time in the electronic gas analysis system was minimal because FO_2 and FCO_2 remained very stable throughout the time (**Table 2**). The highest individual difference in the post-test verification during the non-exercise trials was only of 0.03% in FO_2 and of 0.02% in FCO_2.

Measured and corrected respiratory values

Measured F_EO_2 values reached at $\dot{V}o_{2max}$ during exercise by the young and elderly groups were 17.39 ± 0.29% and 17.18 ± 0.52%, respectively. When these values were corrected with the proposed correction equation, the corresponding F_EO_2 values at $\dot{V}o_{2max}$ were 17.54 ± 0.32% and 17.32 ± 0.53% for the young and elderly groups, respectively. Measured F_ECO_2 values reached at

$\dot{V}o_{2max}$ by the young and elderly groups were 3.77 ± 0.28% and 4.00 ± 0.51%, respectively. When these values were corrected, the corresponding F_ECO_2 values at $\dot{V}o_{2max}$ were 3.80 ± 0.29% and 4.03 ± 0.50% for the young and elderly groups, respectively. Inasmuch as no drift was observed in the calibration factor for ventilation volume during exercise, there were no differences between corrected and measured values of $\dot{V}_{E_{max}}$ in any of the groups (P > 0.05). Average $\dot{V}_{E_{max}}$ was 88% higher (P < 0.001; 95% CI 49 to 80 L·min^{-1}) in the young group compared with the elderly group (137 vs. 73 L·min^{-1}).

Table 2 Calibration (pre-test) and verification (post-test) readings of the exercise and non-exercise trials

	Fractional oxygen concentration (%)				**Fractional carbon dioxide concentration (%)**			
	Room air		**Tank gas**		**Room air**		**Tank gas**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Exercise trials, *n* = 30								
Mean	20.93	20.75**	15.05	14.91**	0.00	0.00**	5.99	5.94**
SD	N/A	0.06	N/A	0.07	N/A	0.01	N/A	0.02
Range								
Min	N/A	20.61	N/A	14.82	N/A	0.00	N/A	5.89
Max	N/A	20.91	N/A	15.05	N/A	0.02	N/A	5.97
Non-exercise trials, *n* = 30								
Mean	20.93	20.93††	15.05	15.05††	0.00	0.01*†	5.99	6.00*††
SD	N/A	0.01	N/A	0.01	N/A	0.01	N/A	0.01
Range								
Min	N/A	20.90	N/A	15.03	N/A	0.00	N/A	5.99
Max	N/A	20.95	N/A	15.07	N/A	0.02	N/A	6.02

Pre, pre-test calibration readings; Post, post-test verification readings.

Significantly different from Pre: * P < 0.01, ** P < 0.001.

Significantly different from the exercise trials: † P < 0.01, †† P < 0.001

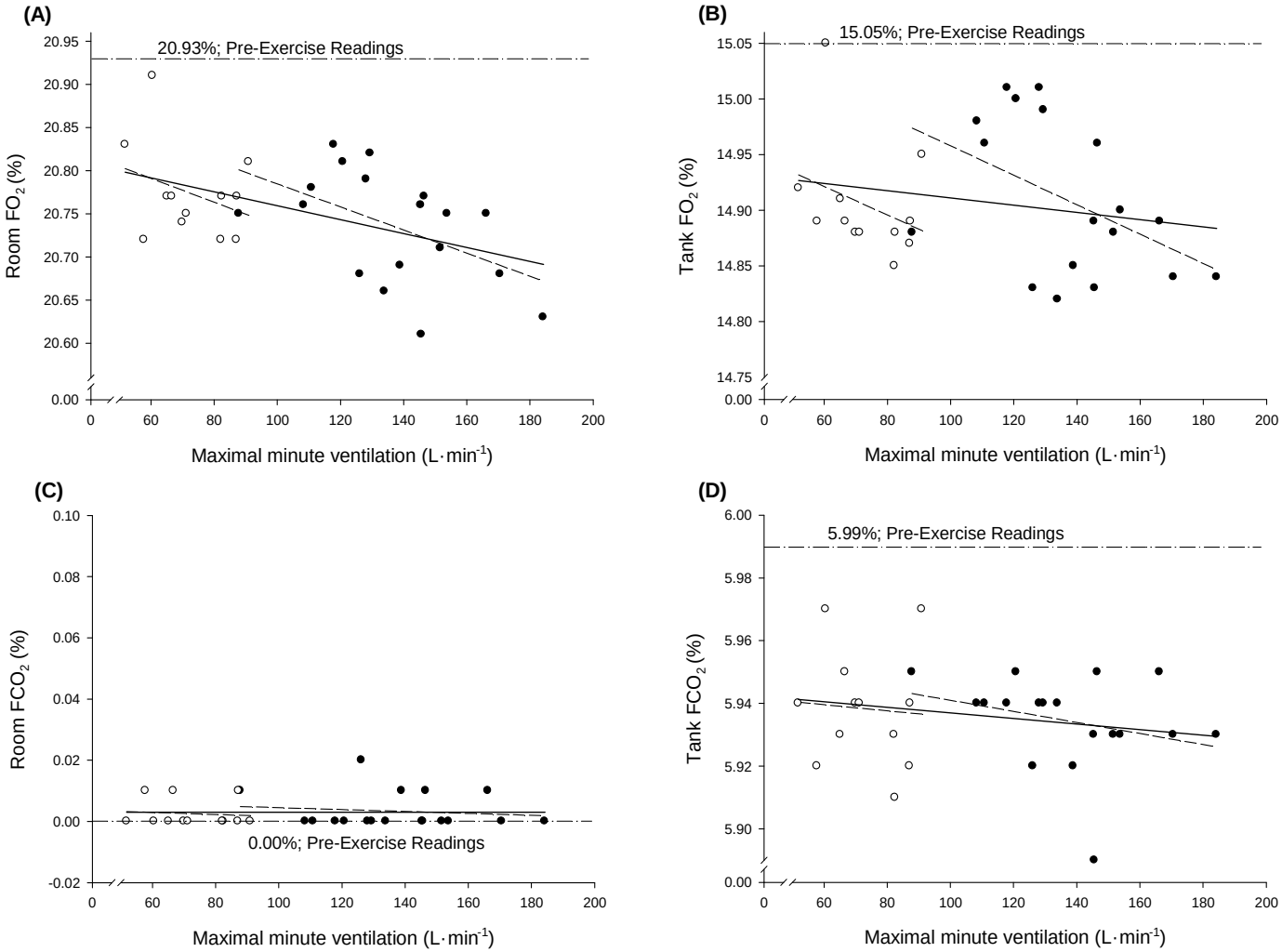

Figure 2: Relationships between the individual values of maximal minute ventilation and the individual post-test verification values of fractional concentrations of oxygen (FO_2; Figures 2A and 2B) and carbon dioxide (FCO_2; Figures2C and 2D) when both calibration gases (room air and tank gas) were run through the metabolic system after maximal exercise. Open circles: elderly sedentary subjects. Filled circles: young athletes.

The corrected F_EO_2 and F_ECO_2 values resulted in systematic significant changes in $\dot{V}co_{2max}$ and $\dot{V}o_{2max}$ values. Measured $\dot{V}co_{2max}$ values reached by the young and elderly groups were 4.93 ± 0.57 L·min^{-1} and 2.82 ± 0.61% L·min^{-1}, respectively. When these values were corrected, the average $\dot{V}co_{2max}$ values (5.07 ± 0.59 L·min^{-1} and 2.98 ± 0.62 L·min^{-1} for the young and elderly groups respectively) were 3-5% higher (P < 0.001) than the corresponding measured values. The measured average $\dot{V}o_{2max}$ values in the young and elderly groups were 4.64 ± 0.56 L·min^{-1} and 2.62 ± 0.50 L·min^{-1}, respectively. Corrected average $\dot{V}o_{2max}$ values (4.35 ± 0.46 and 2.50 ± 0.47 L·min^{-1} for the young and elderly groups, respectively) were 5-6% lower (P < 0.001) than the corresponding measured values. The individual overestimation of the measured $\dot{V}o_{2max}$ values ranged from 0.3% to 11%. **Figure 3A** shows the average and the individual measured and corrected $\dot{V}o_{2max}$ values, expressed relative to kilogram of body mass, in the young and elderly subjects. Average corrected $\dot{V}o_{2max}$ values were 3.6 ml·kg^{-1}·min^{-1} (young) and 1.4 ml·kg^{-1}·min^{-1} (elderly) lower (P < 0.001) than the average measured $\dot{V}o_{2max}$ values. In every subject, the corrected $\dot{V}o_{2max}$ value was lower than the measured value.

Figure 3B shows the average and the individual measured and corrected RER_{max} values in the young and elderly subjects. The average measured RER_{max} values were 1.06 ± 0.05 in the young group and 1.07 ± 0.05 in the elderly group. When these values were corrected, the average RER_{max} values (1.16 ± 0.06 and 1.15 ± 0.06 for the young and elderly groups, respectively) were 8-9% higher (P < 0.001) than the corresponding measured values. In every subject, the corrected RER_{max} value was higher than the measured value.

When the measured values were taken into account, 14 out of the 18 young subjects (78%) and 9 out of the 12 old subjects (75%) satisfied at least two of the criteria established to verify attainment of$\dot{V}o_{2max}$. When the RER_{max} and the $\dot{V}o_{2max}$ values were corrected, the ratio of the subjects who met these criteria increased to 89% and 83% in the young and elderly groups respectively.

Recovery trials

Figure 4 shows the average and individual FO_2 changes observed in 9 subjects when the post-test verification process was repeated several times during the first 30 min of recovery after the completion of the exercise trials, and after disconnecting the gas sample line from the subjects' mask. During the first 5 min of recovery the average FO_2 remained similar to the significantly diminished values (P< 0.001) read immediately after the end of the exercise trials. From that time on, the FO_2 reading values increased progressively and linearly over the time. The disappearance of the drift was completed after 20 min of recovery, although at this time the average FO_2 readings still tended to be slightly lower than the pre-test calibration values (P = 0.20). Similar patterns were observed for the time course of FCO_2 changes (data not shown).

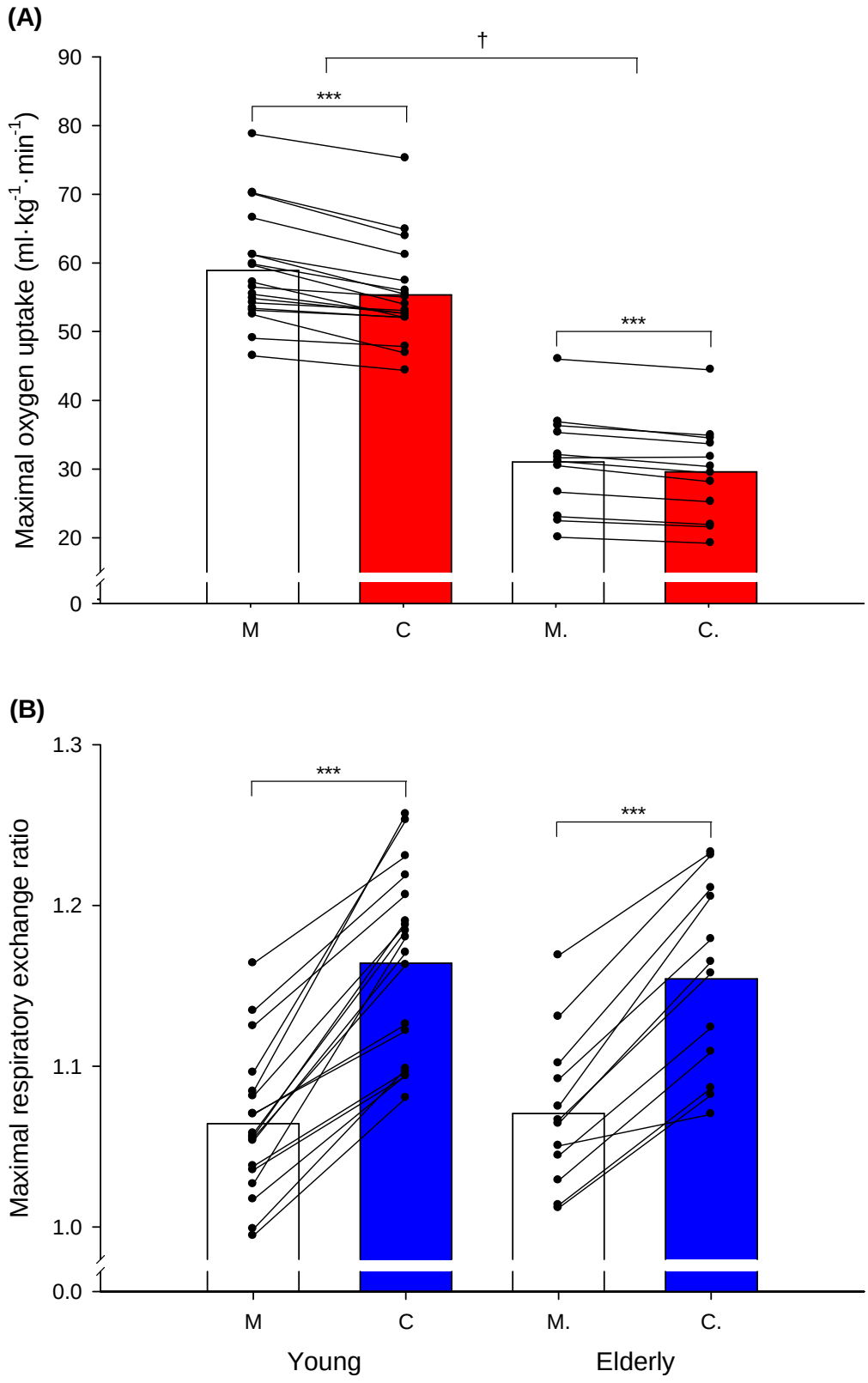

Figure 3: Individual measured (M) and corrected (C) values of maximal oxygen uptake (A) and maximal respiratory exchange ratio (B) in the young and elderly groups. Maximal oxygen uptake is expressed in ml·kg·min^{-1}. The *bars* indicate mean values. *Significant difference between the corrected and the corresponding measured values (P < 0.001). †Significant difference between groups (P < 0.05)

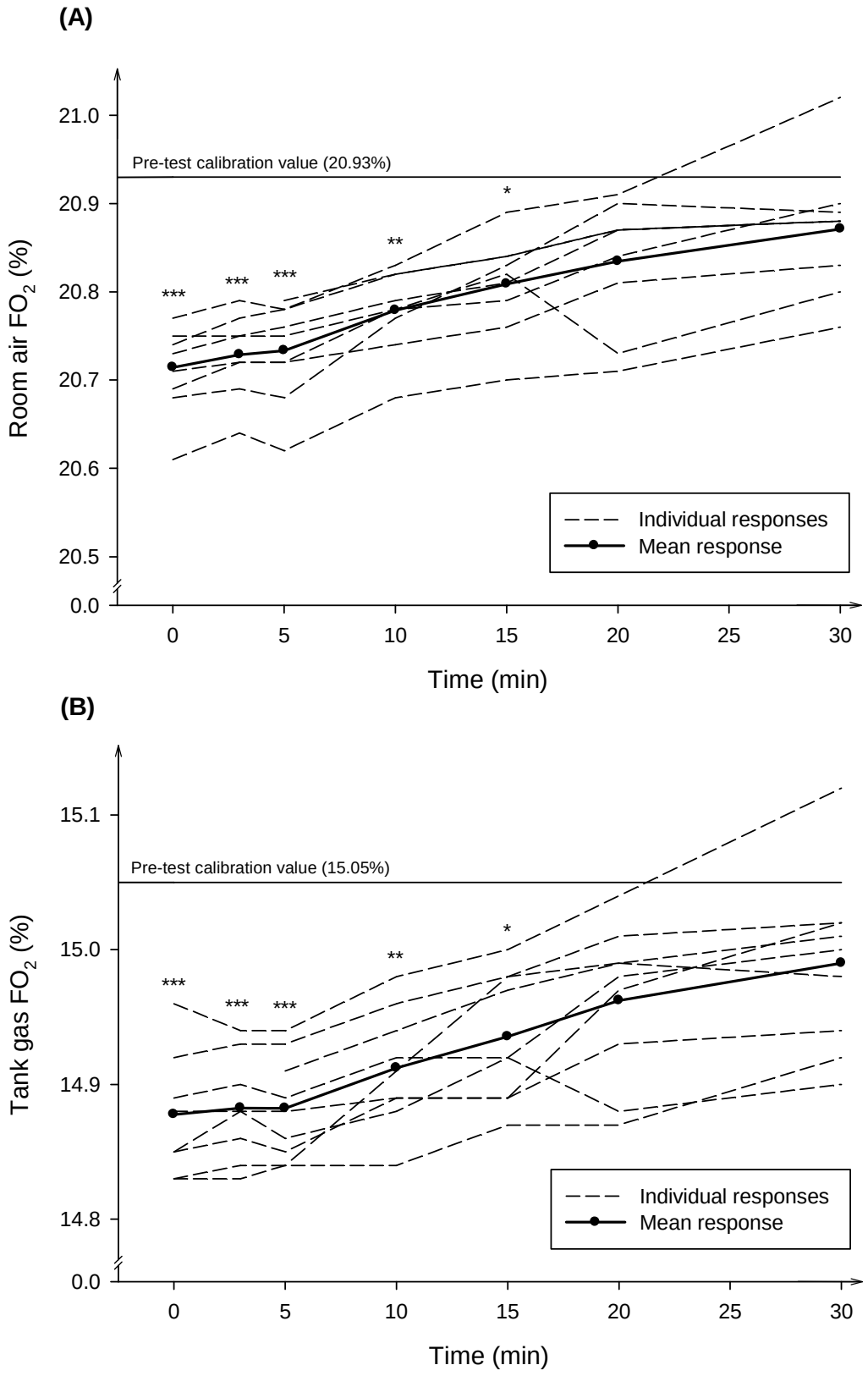

Figure 3: Average and individual time course of the fractional concentrations of oxygen (FO_2) during recovery after maximal exercise. The post-test verification values read by the gas analyzers using the room air (A) and the tank gas (B) were measured at 20 sec, 3, 5, 10, 15, 20 and 30 min of recovery. The number of observations made at each time-point was 9. ***Significantly different from pre-test (P < 0.001); **Significantly different from pre-test (P < 0.01); *Significantly different form pre-test (P < 0.05)

Discussion

The main finding of this study is that the pre-test calibration and the post-test verification values of O_2 and CO_2 demonstrated a downscale drift in the O_2 and CO_2 readings. The drift was observed in all the exercise tests and was higher than the absolute accuracy of at least ± 0.03% (Gore et al., 2013) and ± 0.05% (Jones, 1988) that laboratories should strive to attain for electronic O_2 and CO_2 analyzers. This indicates that the present metabolic system systematically underestimates F_EO_2 and F_ECO_2 values during maximal exercise.

Several potential sources of error, working separately or together, could explain the F_EO_2 and F_ECO_2 downscale drifts during maximal exercise (Robergs et al., 2010). One potential source of error may be due to an electrical instability in the analyzers over time (Kannagi et al., 1983). Evidence of this mechanism, however, has not been provided. When a series of calibrations were assessed during the non-exercise pair-matched trials without any subject being connected to the metabolic system, the O_2 and CO_2 readings remained unchanged over the course of the period (**Table 2**). This suggests that no base-line drift of the analyzers occurred due to an electronic error, indicating that the analyzers were electrically stable for a long period of time.

The most likely factor explaining the reduction in O_2 and CO_2 percentages may be associated with how water vapor is handled in the aspirated gas by the analyzer. The metabolic system used in the present study sends the exhaled gas to the O_2 and CO_2 gas analyzers through a built-in Nafion gas dryer humidifier conducting tube. This tube provides a constant value of water vapor tension of the exhaled and the calibration gases just prior to the entry of the samples into the gas analyzers. It is possible that the observed downscale drift could be partly explained by an incomplete removal of the water vapor tension of the aspirated gas by the analyzer to equilibrate the partial water vapor pressure (PH_2O) into and out of the Nafion tube wall. Since the O_2 and CO_2 analyzers are partial pressure sensors that measure gas fractions of the total gas volume including water vapor, and they are sensitive to the presence of water vapor molecules, the passage of excessive water vapor to the gas analyzers could raise the PH_2O of the sample. A rise in PH_2O would reduce O_2 and CO_2 fractions by the factor $[(P_B\text{-}PH_2O \text{ excess})\cdot(P_B)^{-1}]$ or $[(1\text{-}FH_20)]$ (Gore et al., 2013) and the analyzer would read lower concentration values (Auchincloss, Gilbert, & Baule, 1970). The observation that the O_2 and CO_2 drifts were almost completely reversed in a few min after exercise by simply disconnecting the sampling line from the flow-meter and the subject's mask, and by flushing the system with room air (**Figure 4**), supports the notion that some failure in the drying process occurred during exercise.

Under the assumption of an incomplete removal of the water vapor, it is possible to estimate the average extra amount of PH_2O at a given temperature that was not removed by the Nafion tube to equilibrate the aspirated gas by the analyzers to the level of ambient humidity during exercise. This can be calculated from the average drift values observed in the O_2 (from 20.93% to 20.75% and from 15.05% to 14.91%) and CO_2 analyzers (from 5.99% to 5.94%) (**Table 2**) using the following formula (Gore et al., 2013; Bageant, 1976):

$$\text{Read } O_2\% = [\text{True } O_2\% \cdot (P_B - PH_2O)] \cdot (P_B)^{-1}$$

where read O_2% is the oxygen percentage read during the post-test verification, true O_2% is the oxygen percentage read during the pre-test calibration, and P_B is ambient barometric pressure (in our case: ~724 mmHg).

In that case, the estimated average PH_2O that could not be removed was 6.2 mmHg (range 0.7 to 11 mmHg) for the O_2 calibration with room air, 6.7 mmHg (range: 0-11 mmHg) for the O_2 calibration with the tank, and 6.0 mmHg (range: 2.4-12.1 mmHg) for the CO_2 calibration with the tank. Inasmuch as the PH_2O of the exhaled gas leaving the body is ~ 47 mmHg (on the basis of ~ 100% of relative humidity, at body temperature) (Bageant, 1976), an incomplete average removal of around 6.3 mmHg of water vapor corresponds to ~ 13% of excess in relative humidity ($6.3 \cdot 100 \cdot 47^{-1}$) that cannot be cleared from the circuit, with individual values ranging from 2% to 24%.

The reason why the Nafion tube could not fully equilibrate the gas being conveyed to the analyzers with the ambient humidity is unknown. However it can be related to:

1) A saturation process that reduces active surface area in the Nafion tubing. It is known that some saturation process occurs in the Nafion tubing since the wall of the tubing always retains some residual water, because the sulphonic acid groups within the Nafion polymer will never give up all their water (Mauritz & Moore, 2004). When the dryer becomes progressively physically wet over time, a failure to dry occurs. This failure to dry may be more relevant when the exhaled air flow is high and, therefore, when the aspirated gas sample's flow rate (0.5% of the exhaled flow rate) and its water vapor content are high. For example, in the young exercise trials the amount of water vapor content to be removed out of the Nafion tube can be 16 times higher at maximal exercise (exhaled flow gas: 190 $L \cdot min^{-1}$; aspired gas: 950 $ml \cdot min^{-1}$) than at rest (exhaled flow gas: 12 $L \cdot min^{-1}$; aspired gas: 60 $ml \cdot min^{-1}$). This is in agreement with the significant linear negative correlation observed in this study between $\dot{V}_{E_{max}}$ and the magnitude of the drift in FO_2 (**Figure 2**). This strongly suggests that the higher the $\dot{V}_E$ and the amount of water vapor to be removed, the higher the absolute magnitude of the drift.
2) The inability of the system to maintain a very low water pressure outside, in the air surrounding the Nafion tube wall. An excess of condensate water vapor may be surrounding the Nafion tube as a consequence of the release of the excess of moisture out of the tube. This process may be more pronounced when the Nafion tube is located inside the metabolic measurement cart, such as in the metabolic system used in this study. In such a case, the fan of the metabolic cart cannot remove this excess water vapor condensed inside the metabolic cart.
3) Factors like the accumulation of sweat, saliva, foreign bodies and condensation generated by the subject can enter the internal lumen of the sampling line; a portion of exhaled air is drawn and, therefore, a change in the resistance of the delivery tubing or in the gas sampling rate can occur. This could contribute to a decrease in the gas flow rate and pressure in the sampling tube, leading to irregular results (Atkinson et al., 2005; Gore et al., 2013).

The present results support the above theoretical possibilities that cause an incomplete removal of water vapor of the aspired gas transported from the mouth to the analyzers before the gas

enters the analyzers. This would explain the significant downscale drift in the O_2 and CO_2 analyzers that occurs during continuous measurement during human exercise.

The practical question to consider is the influence of the analyzers' drifts on $\dot{V}o_{2max}$. The correction used for the difference between the pre- and post-test conditions indicated that the corrected $\dot{V}o_{2max}$ values were on average 3.6 ml·kg^{-1}·min^{-1} (young subjects) and 1.4 ml·kg^{-1}·min^{-1} (older subjects) lower than those of the measured values. When expressed relative to the individual $\dot{V}o_{2max}$ values, the average difference between the measured and the corrected $\dot{V}o_{2max}$ values was similar (5-6%) in the young and the elderly subjects. This suggests that, in relative terms, there is a systematic and considerable overestimation in the measurement of $\dot{V}o_{2max}$ that is uniform over a full range of $\dot{V}o_{2max}$ values regardless of exercise duration. The average technological error of 5-6% may be considered unacceptable because it is larger than the ±0.5 to ±3% (technological error) or the ±2.2% to ±4% (technological plus biological variation) accuracy standards accepted for the precision of $\dot{V}o_{2max}$ measurement by most certifying organizations that supervise the accreditation process of the metabolic systems (American Thoracic Society, 1987; Gore et al., 2013). The present results may explain, at least partly, the reason why a measurement error of 5% in $\dot{V}o_{2max}$between laboratories and metabolic systems is nowadays a difficult goal to achieve, owing to the combined technical error and the biological variation (Hodges et al., 2005).

A question raised is the comparison of the corrected $\dot{V}o_{2max}$ and RER_{max} data with published values. When compared to the measured values, the average corrected $\dot{V}o_{2max}$ values in the elderly group (29.6 ml·kg^{-1}·min^{-1}) and the average corrected $\dot{V}o_{2max}$-to cycling work rate values in the young group (14.7 ml O_2·W^{-1}) are lower than the measured values (31.0 ml·kg^{-1}·min^{-1} and 15.7 ml O_2·W^{-1}), and compare favorably with those estimated for the elderly group (29.5 ml·kg^{-1}·min^{-1}) using the formula of the American College of Sports Medicine (2009) and with the average ratio (14.1 ml O_2·W^{-1}; range: 12.1 – 18.6) reported by other investigators using different metabolic systems during long duration (15 – 27 min) incremental maximal cycling tests (Adami, Sivieri, Moia, Perini, & Ferretti, 2013; American College of Sports Medicine, 2009; Armstrong & Costill, 1985; Bowen et al., 2012; Petot, Meilland, Le Moyec L., Mille-Hamard, & Billat, 2012; Pollock et al., 1982; Storer, Davis, & Caiozzo, 1990). The average corrected RER_{max} was 9% higher than the measured RER_{max} in the young group (1.16 vs. 1.06) and 8% higher in the elderly group (1.15 vs. 1.07). When RER_{max} values were not corrected, only 17% of the young and 25% of the elderly subjects reached a RER_{max} greater than 1.10, the most widely used secondary criterion to verify attainment of $\dot{V}o_{2max}$(American College of Sports Medicine, 2009; Howley, Bassett, & Welch, 1995). A major effect of correcting the RER_{max} values was that the ratio of the subjects reaching a RER_{max} greater than 1.10 was increased to 72% in the young group and to 75% in the elderly group. The difference between the corrected and measured RER_{max} values suggests that some inconsistencies and failures found in several studies to satisfy RER_{max} criterion for achievement of $\dot{V}o_{2max}$ may be largely due to an artifact related to technological error (Bowen et al., 2012). This indicates that correction of $\dot{V}o_{2max}$ and RER_{max} values, on the basis of the F_EO_2 and F_ECO_2 drifts observed, produced more reasonable and satisfactory values than the measured ones.

This study has several limitations. The major drawback comes from the fact that we did not corroborate the validity of the correction method suggested. There is also a lack of consensus on

which method is the most appropriate to assess the reliability and validity of $\dot{V}o_2$ measures (Salier et al., 2012). The conventional Douglas bag procedure has been regarded as the gold standard method to validate metabolic measurement systems (McLaughlin et al., 2001; Rietjens et al., 2001). This method remains, however, very limited (Salier et al., 2012). In any case, in close agreement with our corrected values, Medbø et al. (2002) and Larsson et al. (2004) found that a commercial metabolic system (Metamax II), utilizing a built-in Nafion conducting tube, significantly overestimated $\dot{V}o_2$ by 4-13% and underestimated RER by 6% compared to the Douglas bag method. However, other validation studies have produced more varied results (Bassett et al., 2001; McLaughlin et al., 2001; Versteeg & Kippersluis, 1989). An alternative method to validate $\dot{V}o_2$ and RER measures is to use a metabolic calibrator system. However, the external validity of such a test is limited since it often uses dry gases and does not involve challenging factors such as humidified gases and irregular breathing patterns (Macfarlane, 2001). In the absence of a reliable gold standard method, the rationale for the analyzer's drift correction method used in this study is that the time point at $\dot{V}o_{2max}$, which was reached close to the end of the test, is close to the time point at which the post-test verification was undertaken (within 15 sec of the end of each test). It seems, therefore, justifiable to remove and correct the variations observed in F_EO_2 and F_ECO_2 at $\dot{V}o_{2max}$ by adjusting the analyzer pre-exercise base-line values to the post-exercise verification values.

Another limitation of this study is that we used a single metabolic system. Therefore, the generalizability of our findings is constrained to the metabolic cart and the analyzers used. However, four studies reporting the average numerical downscale drifts in O_2 immediately after exercise using other metabolic systems have found values ranging from -0.02% to -0.22% (Armstrong & Costill, 1985; Prieur et al., 1998; Rietjens et al., 2001; Wilmore et al., 1976). This indicates that an absolute downscale drift also occurs in other metabolic systems. If the main source of the error is related to the built-in Nafion gas dryer humidifier conducting tube, a lower error (or none) should occur when gas fractions are measured as fractions of dry gas, when ambient relative humidity is higher than in the present study (e.g. 60%) or when the condensate water vapor surrounding the Nafion tube is more efficiently removed. A wider study is needed to extend the present findings to the wide metabolic systems' population.

Conclusion and perspectives

In conclusion, the present experiment indicates that, under controlled laboratory conditions, a physiologically significant downscale drift in FO_2 and FCO_2 was observed over time at the end of maximal exercise in elderly sedentary and young athletes using a metabolic cart equipped with a built-in Nafion conducting tube. The most likely explanation for the drift is an accumulation of excess water vapor in the sample line which could not be completely removed during transit through the Nafion conducting tube. The correction method proposed indicates that ignoring the effects of the drift would induce an average $\dot{V}o_{2max}$ overestimation of 5-6% and a RER_{max} underestimation of 8-9%, with errors ranging up to 11-12% ($\dot{V}o_{2max}$) and up to 15-16% (RER_{max}). Therefore, ignoring the drift can have an important influence on the accurate calculation of these variables. The disagreement between the measured and the corrected $\dot{V}o_{2max}$ and RER_{max} values observed in this particular metabolic system is not acceptable to test athletes, to prescribe exercise intensities, to calculate the fat oxidation rate from RER values, or to use the respiratory values for some other clinical purposes, such as to guide treatment in patients with chronic heart failure (Bowen et al., 2012), to enter in cardiac transplantation listing, to indicate the health status or to predict prognosis and mortality (Mehra et al., 2006; Myers et al., 2002). The implications of the present study point to the necessity to check FO_2 and FCO_2 values by carefully calibrating the pre-test calibration gases and verifying a possible shift immediately after exercise, as well as to correct the respiratory data in situations where the drift in O_2 and CO_2 analyzers occurs. Special care must be taken in studies where a Nafion conducting tube is used. Further research in this area is certainly warranted to establish valid correction factors for each device.

Author Contributions

EG, IG and JE conceived and designed the experiments; EG, IG and JA contributed to the acquisition and analysis of the data; EG, IG, JE and JA interpreted the data; EG wrote the first draft; EG, IG, JE and JA critically reviewed and edited the drafts; all authors approved the final version of the manuscript.

Acknowledgements

We gratefully thank Irene Madariaga for her professional assistance in exercise testing.

References

Adami, A., Sivieri, A., Moia, C., Perini, R., & Ferretti, G. (2013). Effects of step duration in incremental ramp protocols on peak power and maximal oxygen consumption. *Eur J Appl Physiol, 113,* 2647-2653.

American College of Sports Medicine (2009). *ACSM's guidelines for exercise testing and prescription*. (8 ed.) Philadelphia: Lippincott Williams & Wilkins.

American Thoracic Society (1987). Standardization of spirometry: 1987 update. Statement of the American Thoraccic Society. *Am.Rev.Respir.Dis, 136,* 1285-1298.

Armstrong, L. E. & Costill, D. L. (1985). Variability of respiration and metabolism: responses to submaximal cycling and running. *Res Q Exerc Sport, 56,* 93-96.

Atkinson, G., Davison, R. C., & Nevill, A. M. (2005). Performance characteristics of gas analysis systems: what we know and what we need to know. *Int.J Sports Med., 26 Suppl 1,* S2-10.

Auchincloss, J. H. Jr., Gilbert, R., & Baule, G. H. (1970). Control of water vapor during rapid analysis of respiratory gases in expired air. *J Appl Physiol, 28,* 245-247.

Bageant, R. A. (1976). Oxygen analyzers. *Respir.Care, 21,* 410-416.

Bassett, D. R. Jr., Howley, E. T., Thompson, D. L., King, G. A., Strath, S. J., McLaughlin, J. E. et al. (2001). Validity of inspiratory and expiratory methods of measuring gas exchange with a computerized system. *J Appl Physiol (1985.), 91,* 218-224.

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1973). On-line computer analysis and breath-by-breath graphical display of exercise function tests. *J Appl.Physiol, 34,* 128-132.

Bowen, T. S., Cannon, D. T., Begg, G., Baliga, V., Witte, K. K., & Rossiter, H. B. (2012). A novel cardiopulmonary exercise test protocol and criterion to determine maximal oxygen uptake in chronic heart failure. *J Appl Physiol (1985.), 113,* 451-458.

Day, J. R., Rossiter, H. B., Coats, E. M., Skasick, A., & Whipp, B. J. (2003). The maximally attainable VO2 during exercise in humans: the peak vs. maximum issue. *J Appl Physiol (1985.), 95,* 1901-1907.

Gore, C. J., Clark, R. J., Shipp, N. J., van der Ploeg, G. E., & Withers, R. T. (2003). CPX/D underestimates VO(2) in athletes compared with an automated Douglas bag system. *Med.Sci.Sports Exerc, 35,* 1341-1347.

Gore, C. J., Tanner, R. K., Fuller, K. L., & Stanef, F. (2013). Determination of maximal oxygen consumption (VO2max). In R.K.Tanner & C. J. Gore (Eds.), *Physiological tests for elite athletes* (pp. 103-122). Champaign, IL: Human Kinetics.

Hodges, L. D., Brodie, D. A., & Bromley, P. D. (2005). Validity and reliability of selected commercially available metabolic analyzer systems. *Scand.J.Med.Sci.Sports, 15,* 271-279.

Howley, E. T., Bassett, D. R. Jr., & Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Med.Sci.Sports Exerc., 27,* 1292-1301.

Jones, N. L. (1988). *Clinical exercise testing*. (3 ed.) Philadelphia: W.B. Saunders.

Kannagi, T., Bruce, R. A., Hossack, K. F., Chang, K., Kusumi, F., & Trimble, S. (1983). An evaluation of the Beckman Metabolic Cart for measuring ventilation and aerobic requirements during exercise. *J Cardiac Rehabil, 3,* 38-53.

Larsson, P. U., Wadell, K. M., Jakobsson, E. J., Burlin, L. U., & Henriksson-Larsen, K. B. (2004). Validation of the MetaMax II portable metabolic measurement system. *Int.J Sports Med., 25,* 115-123.

Macfarlane, D. J. (2001). Automated metabolic gas analysis systems: a review. *Sports Med., 31,* 841-861.

Mauritz, K. A. & Moore, R. B. (2004). State of understanding of nafion. *Chem.Rev., 104,* 4535-4585.

McLaughlin, J. E., King, G. A., Howley, E. T., Bassett, D. R., Jr., & Ainsworth, B. E. (2001). Validation of the COSMED K4 b2 portable metabolic system. *Int.J Sports Med., 22,* 280-284.

Medbø, J. I., Mamen, A., Welde, B., von Heimburg E., & Stokke, R. (2002). Examination of the Metamax I and II oxygen analysers during exercise studies in the laboratory. *Scand.J Clin.Lab Invest, 62,* 585-598.

Mehra, M. R., Kobashigawa, J., Starling, R., Russell, S., Uber, P. A., Parameshwar, J. et al. (2006). Listing criteria for heart transplantation: International Society for Heart and Lung Transplantation guidelines for the care of cardiac transplant candidates--2006. *J Heart Lung Transplant., 25,* 1024-1042.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, J. E. (2002). Exercise capacity and mortality among men referred for exercise testing. *N.Engl.J Med., 346,* 793-801.

Petot, H., Meilland, R., Le Moyec L., Mille-Hamard, L., & Billat, V. L. (2012). A new incremental test for VO(2)max accurate measurement by increasing VO(2)max plateau duration, allowing the investigation of its limiting factors. *Eur J Appl Physiol, 112,* 2267-2276.

Pollock, M. L., Foster, C., Schmidt, D., Hellman, C., Linnerud, A. C., & Ward, A. (1982). Comparative analysis of physiologic responses to three different maximal graded exercise test protocols in healthy women. *Am Heart J, 103,* 363-373.

Prieur, F., Busso, T., Castells, J., Bonnefoy, R., Benoit, H., Geyssant, A. et al. (1998). Validity of oxygen uptake measurements during exercise under moderate hyperoxia. *Med.Sci.Sports Exerc., 30,* 958-962.

Rietjens, G. J., Kuipers, H., Kester, A. D., & Keizer, H. A. (2001). Validation of a computerized metabolic measurement system (Oxycon-Pro) during low and high intensity exercise. *Int.J Sports Med., 22,* 291-294.

Robergs, R. A., Dwyer, D., & Astorino, T. (2010). Recommendations for improved data processing from expired gas analysis indirect calorimetry. *Sports Med., 40,* 95-111.

Salier, E. J., Rosdahl, H., & Schantz, P. (2012). Validity of the Oxycon Mobile metabolic system under field measuring conditions. *Eur.J Appl.Physiol, 112,* 345-355.

Storer, T. W., Davis, J. A., & Caiozzo, V. J. (1990). Accurate prediction of VO2max in cycle ergometry. *Med.Sci Sports Exerc, 22,* 704-712.

Taylor, H., Buskirk, E., & Henschel, A. (1955). Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl.Physiol, 8,* 73-80.

Versteeg, P. G. & Kippersluis, G. J. (1989). Automated systems for measurement of oxygen uptake during exercise testing. *Int.J Sports Med., 10,* 107-112.

Wasserman, K., Hansen, J. E., Sue, D. Y., Whipp, B. J., & Casaburi, R. (1994). Appendix D. Calculations, formulas, and examples. In K.Wasserman, J. E. Hansen, D. Y. Sue, B. J. Whipp, & R. Casaburi (Eds.), *Principles of exercise testing and interpretation* (4 ed., pp. 454-464). Philadelphia: Williams & Wilkins.

Wasserman, K., Hansen, J. E., Sue, D. Y., Whipp, B. J., & Casaburi, R. (1994). Appendix F. Tables and nomogram. In K.Wasserman, J. E. Hansen, D. Y. Sue, B. J. Whipp, & R. Casaburi (Eds.), *Principles of exercise testing and interpretation* (4 ed., pp. 468-470). Philadelphia: Williams & Wilkins.

Wilmore, J. H., Davis, J. A., & Norton, A. C. (1976). An automated system for assessing metabolic and respiratory function during exercise. *J Appl.Physiol, 40,* 619-624.

Winter, E. M. (2012). Calibration and verification of instruments. *J Sports Sci., 30,* 1197-1198.

Chapter 3

Heart rate-based prediction of fixed blood lactate thresholds in professional team-sport players

* Garcia-Tabar I, Llodio I., Sánchez-Medina L, Ruesta M, Ibañez J & Gorostiaga EM. Heart rate-based prediction of fixed blood lactate thresholds in professional team-sport players. *Journal of Strength and Conditioning Research* 2015;29(10):2794-2801.

Heart rate based prediction of fixed blood lactate thresholds in professional team-sport players

Authors

Ibai Garcia-Tabar[1], Iñaki Llodio[2], Luis Sánchez-Medina[1],Maite Ruesta[1],Javier Ibañez[1] and Esteban M. Gorostiaga[1]

Affiliations

[1]Studies, Research and Sports Medicine Center, Government of Navarre, Pamplona, Spain

[2]Department of Physical Activity and Sport Sciences, University of the Basque Country, Vitoria-Gasteiz, Spain

Abstract

The aim of this study was to investigate whether the speed associated with 90% of maximal heart rate (S90%HR_{max}) could predict speeds at fixed blood lactate concentrations of 3 mmol·L^{-1} (S3mM) and 4 mmol·L^{-1} (S4mM). Professional team-sport players of futsal (n = 10), handball (n = 16) and basketball (n = 10) performed a four-stage discontinuous progressive running test followed, if exhaustion was not previously achieved, by an additional maximal continuous incremental running test to attain maximal heart rate (HR_{max}). The individual S3mM,S4mM and S90%HR_{max} were determined by linear interpolation. S3mM (11.6 ± 1.5 km·h^{-1}) and S4mM (12.5 ± 1.4 km·h^{-1}) did not differ (p> 0.05) from S90%HR_{max} (12.0 ± 1.2 km·h^{-1}). Very large significant (p < 0.001) relationships were found between S90%HR_{max} and S3mM (r = 0.82; *SEE* = 0.87 km·h^{-1}), as well as between S90%HR_{max} and S4mM (r = 0.82; *SEE* = 0.87 km·h^{-1}). S3mM and S4mM inversely correlated with %HR_{max} associated with running speeds of 10 and 12 km·h^{-1} (r = 0.78 - 0.81; p < 0.001; *SEE* = 0.94 - 0.87 km·h^{-1}). In conclusion, S3mM and S4mM can be accurately predicted by S90%HR_{max} in professional team-sport players.

Keywords: OBLA, maximal lactate steady state, exercise testing, elite athletes, heart rate monitor

Introduction

Competitive team-sports such as handball, basketball and futsal are high-intensity intermittent team-sports that place heavy emphasis on aerobic fitness. Even though team sports are not endurance sports *per se*, a minimum level of aerobic fitness is crucial in the ability to maintain an elevated intensity work in order to play at top level professional leagues (Alvarez, D'Ottavio, Vera, & Castagna, 2009; Gorostiaga, Granados, Ibanez, & Izquierdo, 2005; Povoas et al., 2012; Ziv & Lidor, 2009). Aerobic improvement increases the number of sprints and the distance covered during a match, and promotes more ball involvement in soccer (Helgerud, Engen, Wisloff, & Hoff, 2001). Reduction of the time spent at high-intensity during the games observed in handball, basketball and futsal (Alvarez et al., 2009; Ben Abdelkrim, El Fazaa, & El Ati, 2007; Povoas et al., 2012) also indicates the potential benefit of aerobic conditioning in indoor team-sports. Therefore, it seems of paramount importance to frequently assess changes in aerobic capacity in professional team-sport players throughout the season.

The speed at the "maximal lactate steady state" (MLSS) is generally considered the gold standard for determination of aerobic capacity. However, MLSS determination is tedious and time consuming (Mann, Lamberts, & Lambert, 2013). In field testing of team-sports, fixed lactate thresholds, such as running speeds associated with 3 $mmol \cdot L^{-1}$ (S3mM) and 4 $mmol \cdot L^{-1}$ (S4mM), which is also termed OBLA (Sjödin & Jacobs, 1981), are often preferred to MLSS (Gorostiaga, Granados, Ibanez, Gonzalez-Badillo, & Izquierdo, 2006; Granados, Izquierdo, Ibanez, Ruesta, & Gorostiaga, 2008; Loures et al., 2015; McMillan et al., 2005) because they reduce the time and cost of the assessment procedure, are easy to measure in several athletes at the same time in field settings, and have been shown to reflect the speed at the MLSS as appropriate as other lactate thresholds (Beneke, 1995; Hauser, Adam, & Schulz, 2014). Unfortunately, the determination of the fixed lactate thresholds requires qualified personnel and involves blood sampling, which is an invasive technique that can be aversive to some participants. Moreover, when performing field testing of teams, typically composed of 10-25 players, the cost of blood sampling is high and requires the participation of several qualified professionals. These issues often hinder the appropriate monitoring of endurance capacity in team-sports.

In an attempt to monitor players and predict aerobic performance more regularly, sub-maximal non-invasive low-cost tests have been of general interest to sport teams and to the sport scientist's community. Based on previous studies (Garcia-Tabar et al., 2013; Kindermann, Simon, & Keul, 1979; McMillan et al., 2005; Mujika & Padilla, 2001; Mujika, 2012) and personal observations from years of professional experience in the assessment of team-sports' endurance (Gorostiaga et al., 2006; Gorostiaga et al., 2009; Granados et al., 2008), we have noticed that the S3mM and S4mM determined during a progressive maximal field test usually occur at a mean intensity close to 90% of maximal heart rate (HR_{max}). It also seems that this relationship is maintained despite alterations in the intensity of the individual or fixed lactate thresholds due to training, detraining or hypoxia (Foster, Fitzgerald, & Spatz, 1999; Helgerud et al., 2001; McMillan et al., 2005; Mujika, 2012; Friedmann, Bauer, Menold, & Bartsch, 2004; Hurley et al., 1984; Lucia, Hoyos, Perez, & Chicharro, 2000). Nevertheless, whether the intensity at 90%HR_{max} could be used as a simple variable to assess S3mM and S4mM has never been investigated. Being able to assess aerobic capacity by means of an easy and non-invasive estimation of the fixed lactate thresholds would certainly cheapen and facilitate the monitoring of aerobic performance. This would be of

particular interest to teams and coaches with limited resources. Accordingly, the primary aim of the current study was to investigate the relationships between the running speeds associated with the broadly used 90% of HR_{max} ($S90\%HR_{max}$) and the S3mM and S4mM (or OBLA). It was hypothesized that $S90\%HR_{max}$ would be related to fixed lactate thresholds in team-sport players.

Methods

Experimental Approach to the Problem

A cross-sectional study was carried out to investigate the relationships between S90%HR_{max} and fixed blood lactate thresholds (S3mM and S4mM). Professional team-sport players performed a four-stage discontinuous progressive running test followed, if exhaustion was not previously achieved, by an additional maximal continuous incremental running test to attain HR_{max}. The individual S3mM, S4mM and S90%HR_{max} were determined by linear interpolation and relationships examined.

Subjects

Players from three professional teams of futsal (n = 10), handball (n = 16) and basketball (n = 10) participated in this study. The teams belonged to the Spanish First Divisions of futsal and handball, and the Spanish National Second Division of basketball. All participants were free of known cardiovascular, respiratory and circulatory dysfunctions, and they were not taking any drug or medication known to influence physical performance. Please see Table 1 for participant characteristics.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Local Institutional Review Board. Athletes and coaches were carefully informed about the possible risks and benefits of the project, and written informed consent was obtained from every volunteer.

Table 1 Anthropometric characteristics of the studied professional sport teams (n = 36)

	Handball team (n = 16)	**Basketball team (n = 10)**	**Futsal team (n = 10)**
Age (years)	26 ± 5	26 ± 4	24 ± 5
Body mass (kg)	90.1 ± 9.2*	93.4 ± 11.8*	72.8 ± 4.9
Height (cm)	189.1 ± 7.6*	194.2 ± 7.8*	177.4 ± 5.7
Body mass index ($kg \cdot m^{-2}$)	25.1 ± 1.5*	24.6 ± 2.0	23.1 ± 1.4

*Significantly different from futsal ($p < 0.05$)

Procedures

The study was conducted during the first 2-3 days of the pre-season training period, i.e. 4-6 weeks before the start of official competitions. Testing was integrated into the training schedule. Participants refrained from vigorous exercise during the previous 24 h and were instructed to fast for at least 2 h before the exercise test. They were also instructed to abstain from caffeinated and alcoholic beverages during the testing day. The pre-exercise meal for each participant was the same in an effort to standardize nutritional intake. All testing sessions were carried out in the same indoor court and at the same time of the day to lessen circadian variability.

After a non-standardized 15-min warm-up period, participants performed a four-stage discontinuous progressive running test around the indoor court (40x20 m) (Gorostiaga et al., 2005; Gorostiaga et al., 2006). Each stage was 5 min long, with a 3 min resting period between stages. The running speeds at each stage were 10, 12, 14 and 16 $km \cdot h^{-1}$. This incremental test protocol was the test protocol routinely used for regular testing by all these three sport teams. The choice of the running speeds was made to assure blood lactate concentration ($[La^-]$) values lower and similar or slightly higher than 4 $mmol \cdot L^{-1}$, based on previous endurance assessments performed for exercise prescription purposes with these teams (Gorostiaga et al., 2005; Gorostiaga et al., 2006).Five min long stage duration protocol was used because it is the minimum stage duration needed to reach a lactate equilibrium between muscle and blood at exercise intensities approaching S4mM (Rusko et al., 1986), and because S4mM approximates MLSS better with 5 min stage duration protocols than with lower stage duration protocols (Kuipers, Rietjens, Verstappen, Schoenmakers, & Hofman, 2003). To ensure a constant speed during each stage, participants were instructed to even pace their running following an audio signal connected to a pre-programmed laptop (Balise Temporelle, Bauman, Switzerland). Heart rate (HR) was recorded every 15 s (Polar Electro Oy, Sport Tester, Kempele, Finland) and averaged for the last 3 min of every completed stage. Immediately after each stage, and until participants attained a $[La^-]$ above 5 $mmol \cdot L^{-1}$, capillary blood samples from an hyperaemic earlobe were taken and $[La^-]$ amperometrically determined (Arkray KDK Corporation, Lactate Pro LT-1710, Shiga, Japan).

The participants (3 futsal players) who did not reach volitional exhaustion during the discontinuous running test were required to rest for 5-8 min after the completion of the final stage, and then began a maximal continuous incremental test to obtain their HR_{max}. Starting speed in this additional test was 13.6 $km \cdot h^{-1}$, and it was increased by 0.8 $km \cdot h^{-1}$ every min until players could no longer maintain the required speed. Either in the discontinuous or continuous protocol, volunteers were vigorously encouraged to complete exhaustion. HR_{max} was considered to be the highest HR value recorded, and it coincided in most cases with the voluntary termination of exercise (Nes, Janszky, Wisloff, Stoylen, & Karlsen, 2013).

S3mM and S4mM were determined by linear interpolation. $S90\%HR_{max}$ was calculated following identical procedures. The test-retest intraclass correlation coefficients of S3mM, S4mM and HR_{max} have been shown to range between 0.94 and 0.99, and coefficients of variation (CV) between 1.2 and 1.7% (Borch, Ingjer, Larsen, & Tomten, 1993; Weltman et al., 1990; Pfitzinger & Freedson, 1998).

Statistical Analyses

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, USA). Gaussian distribution was verified by Shapiro-Wilk's test when appropriate. Multiple comparisons between teams were evaluated using the Kruskal-Wallis test. Linear regression analyses were performed to determine the relationships between the variables of interest. Assumptions of linear regressions were checked and met. Evaluation of Cook's Distance revealed minimal influence of the individual data points on the regression models. Pearson product-moment correlation coefficients (*r*) were used to indicate the magnitude and direction of each linear relationship. The magnitudes of the correlations were interpreted as follows: 0.1-0.3 small, 0.3-0.5 moderate, 0.5-0.7 large, 0.7-0.9

very large and > 0.9 extremely large (Hopkins, Marshall, Batterham, & Hanin, 2009). The accuracy of each linear regression was evaluated using the standard error of the estimates (*SEE*), the 95% confidence intervals (CI) for the slope and the 90% CI for the correlation coefficients. The slopes of the regression lines were compared using analysis of covariance (ANCOVA). *Post hoc* power calculation for the linear regressions, assuming type I error of 0.05, indicated a power of above 99%. Statistical significance was set at $p < 0.05$. Data in the text, tables and figures are reported as mean and standard deviation (SD).

Results

Figure 1 illustrates [La^-] and %HR_{max} at the 10, 12 and 14 km·h^{-1} exercise stages. Absolute HR values did not differ among the teams at any of the exercise stages (p >0.05). HR values for all the teams as a whole were 156 ± 10, 171 ± 10 and 182 ± 9 b·min^{-1} at completion of the 10, 12 and 14 km·h^{-1} stages, respectively. Basketball players reached significantly lower HR_{max} values (184 ± 9 b·min^{-1}) compared to futsal (192 ± 5 b·min^{-1}; p = 0.038; 95%CI -14.60 to -0.33) and handball (192 ± 6 b·min^{-1}; p = 0.046; 95%CI -16.71 to -0.10) players. Every participant fulfilled the criterion of HR_{max} above 90% of age-predicted HR_{max} (Nes et al., 2013).

Descriptive features of S3mM, S4mM and S90%HR_{max} are summarized in Table 2. Mean S3mM significantly differed from mean S4mM (p = 0.027; 95% CI -1.75 to -0.08). There were no significant differences between mean S90%HR_{max} and mean S3mM (p = 0.51; 95% CI -1.20 to 0.38). Mean S90%HR_{max} neither differed from mean S4mM (p = 0.32; 95% CI -0.28 to 1.29).

Figure 2 shows the linear relationships between S90%HR_{max} and S3mM (Figure 2A), as well as between S90%HR_{max} and S4mM (Figure 2B). The rate of increase did not differ between the basketball, handball and futsal teams (p >0.05). Regression equations of Figure 2 are reported in Table 3.

Unplanned regression analyses revealed significant linear relationships between %HR_{max} at 10 and 12 km·h^{-1} and S3mM and S4mM (Figure 3). There were no differences in the rate of decline between the teams (p >0.05). Very large and significant (p <0.001) correlations between S3mM and %HR_{max} at S3mM (r = 0.67; SEE = 1.14 km·h^{-1}; 95% CI 0.16 to 0.39; 90% CI ±0.16), and between S4mM and %HR_{max} at S4mM (r = 0.68; SEE = 1.09 km·h^{-1}; 95% CI 0.14 to 0.34; 90% CI ±0.16) were also found.

Speed at 90% HR_{max}

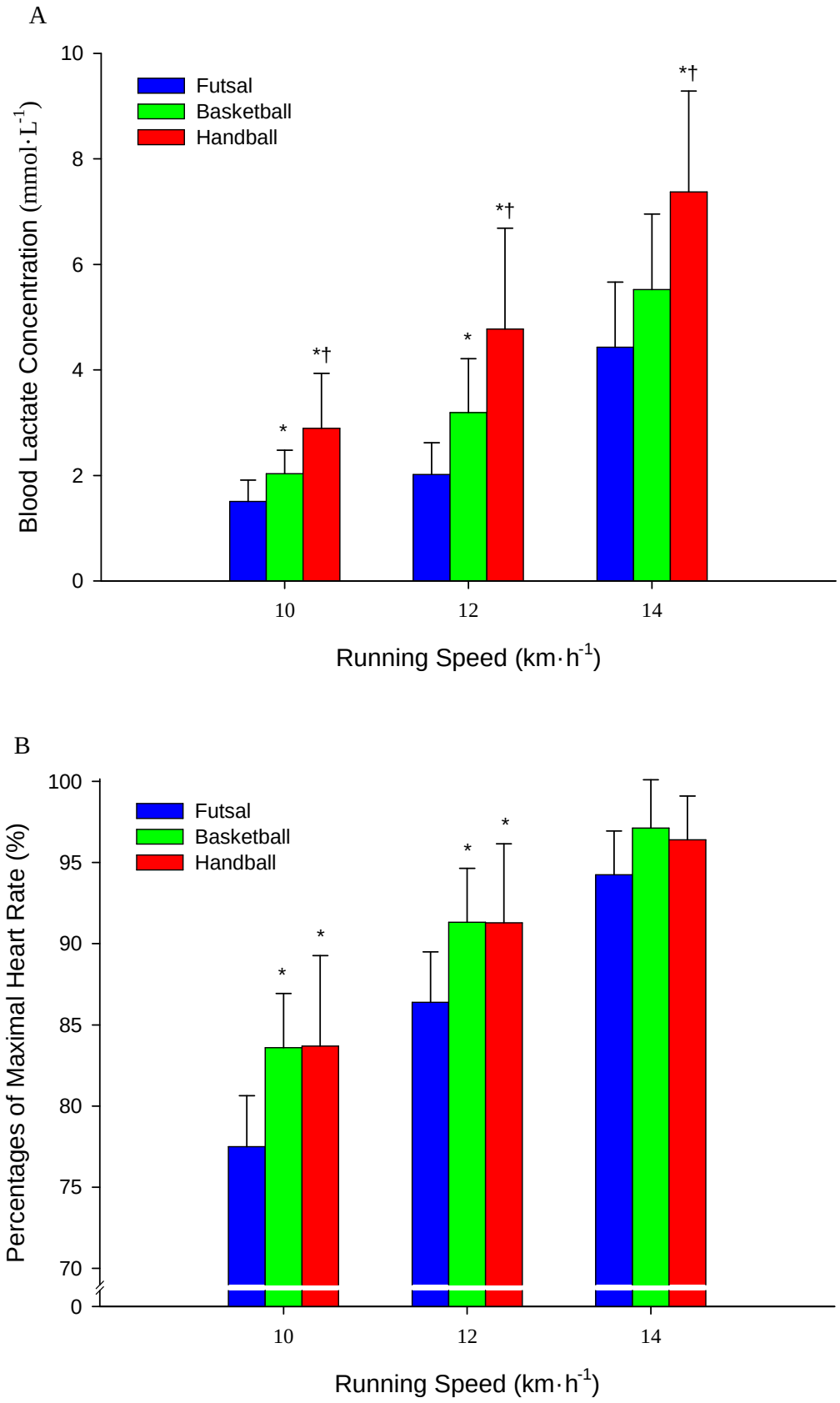

Figure 1: Mean (SD) blood lactate concentrations (A) and percentages of maximal heart rate (B) at 10, 12 and 14 km·h^{-1} stages for each sport team. *Significantly different from futsal ($p < 0.05$); †Significantly different from basketball ($p < 0.05$)

Table 2. Descriptive features of the running speeds at blood lactate concentrations ($[La^-]$) of 3mmol·L^{-1} (S3mM), 4mmol·L^{-1} (S4mM) and 90% of maximal heart rate (S90%HR_{max})

	S3mM			S4mM			S90%HR_{max}		
	Speed (km·h^{-1})	**HR (b·min^{-1})**	**%HR_{max} (%)**	**Speed (km·h^{-1})**	**HR (b·min^{-1})**	**%HR_{max} (%)**	**Speed (km·h^{-1})**	**HR (b·min^{-1})**	**$[La^-]$ (mmol·L^{-1})**
Handball Team	10.5 ± 1.2*†	163 ± 7*	85.1 ± 3.0*†	11.5 ± 1.2*†	169 ± 9*	89.3 ± 4.2*†	11.5 ± 1.3*	173 ± 5	4.3 ± 1.1*†
Basketball Team	11.8 ± 1.1*	164 ± 7*	90.1 ± 2.9	12.8 ± 1.0	171 ± 8*	93.5 ± 2.8	11.7 ± 1.0*	166 ± 8	3.1 ± 0.7
Futsal Team	13.0 ± 0.7	175 ± 6	90.4 ± 1.2	13.7 ± 0.7	181 ± 6	93.2 ± 1.2	12.9 ± 0.7	173 ± 5	2.9 ± 0.3
Mean	11.6 ± 1.5‡	167 ± 8‡	87.9 ± 3.6	12.5 ± 1.4	173 ± 9	91.0 ± 4.1	12.0 ± 1.2	171 ± 7	3.6 ± 1.0

*Significantly different from futsal ($p < 0.05$); †Significantly different from basketball ($p < 0.05$); ‡Significantly different from S4mM ($p < 0.05$)

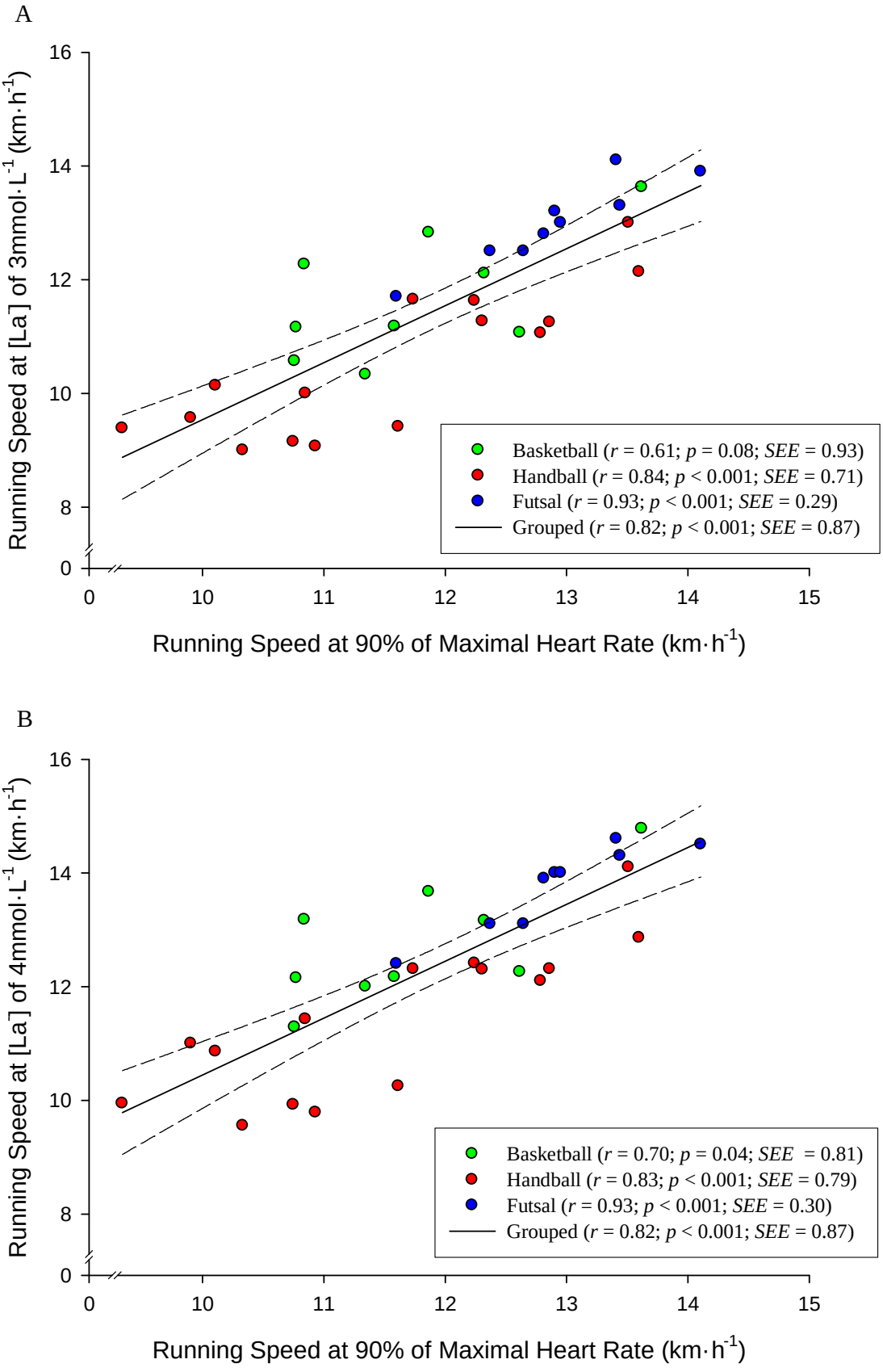

Figure 2: Relationships between the running speed at 90% of maximal heart rate and the running speeds at blood lactate concentration ($[La^-]$) of 3 $mmol \cdot L^{-1}$ (A) and 4 $mmol \cdot L^{-1}$ (B). Grouped linear regressions solid lines (-); 95% confidence intervals dashed lines (- -)

Table 3. Linear regression equations for prediction of the running speeds at [La^-] of 3 (S3mM) and 4 $mmol \cdot l^{-1}$ (S4mM) by the running speed at 90% of maximal heart rate (S90%HR_{max})

		n	*r*	r^2	*SEE*	*p*	Regression Equation	95% CI	90% CI
S3mM	Basketball	10	0.61	0.37	0.93	0.08	S3mM = 0.6893(S90%HR_{max}) +3.5857	-0.11 to 1.50	±0.39
	Handball	16	0.84	0.71	0.71	< 0.001	S3mM = 0.8024(S90%HR_{max}) +1.2681	0.49 to 1.11	±0.14
	Futsal	10	0.93	0.87	0.29	< 0.001	S3mM = 0.9647(S90%HR_{max}) + 0.5387	0.63 to 1.32	±0.10
	Grouped	36	0.82	0.67	0.87	< 0.001	S3mM = 1.0013(S90%HR_{max}) - 0.4698	0.74 to 1.26	±0.10
S4mM	Basketball	10	0.70	0.49	0.81	0.04	S4mM = 0.7623(S90%HR_{max}) + 3.784	0.06 to 147	±0.33
	Handball	16	0.83	0.69	0.79	< 0.001	S4mM = 0.8339(S90%HR_{max}) + 1.798	0.49 to 1.17	±0.15
	Futsal	10	0.93	0.87	0.30	< 0.001	S4mM = 0.9622(S90%HR_{max}) + 1.337	0.62 to 1.33	±0.10
	Grouped	36	0.82	0.67	0.87	< 0.001	S4mM = 0.9998(S90%HR_{max}) + 0.4512	0.74 to 1.26	±0.10

[La^-], blood lactate concentration; *SEE*, standard error of the estimate; 95% CI, 95% confidence intervals for the slope; 90% CI, 90% confidence intervals for the Pearson product-moment correlation coefficients (*r*)

Speed at 90% HR_{max}

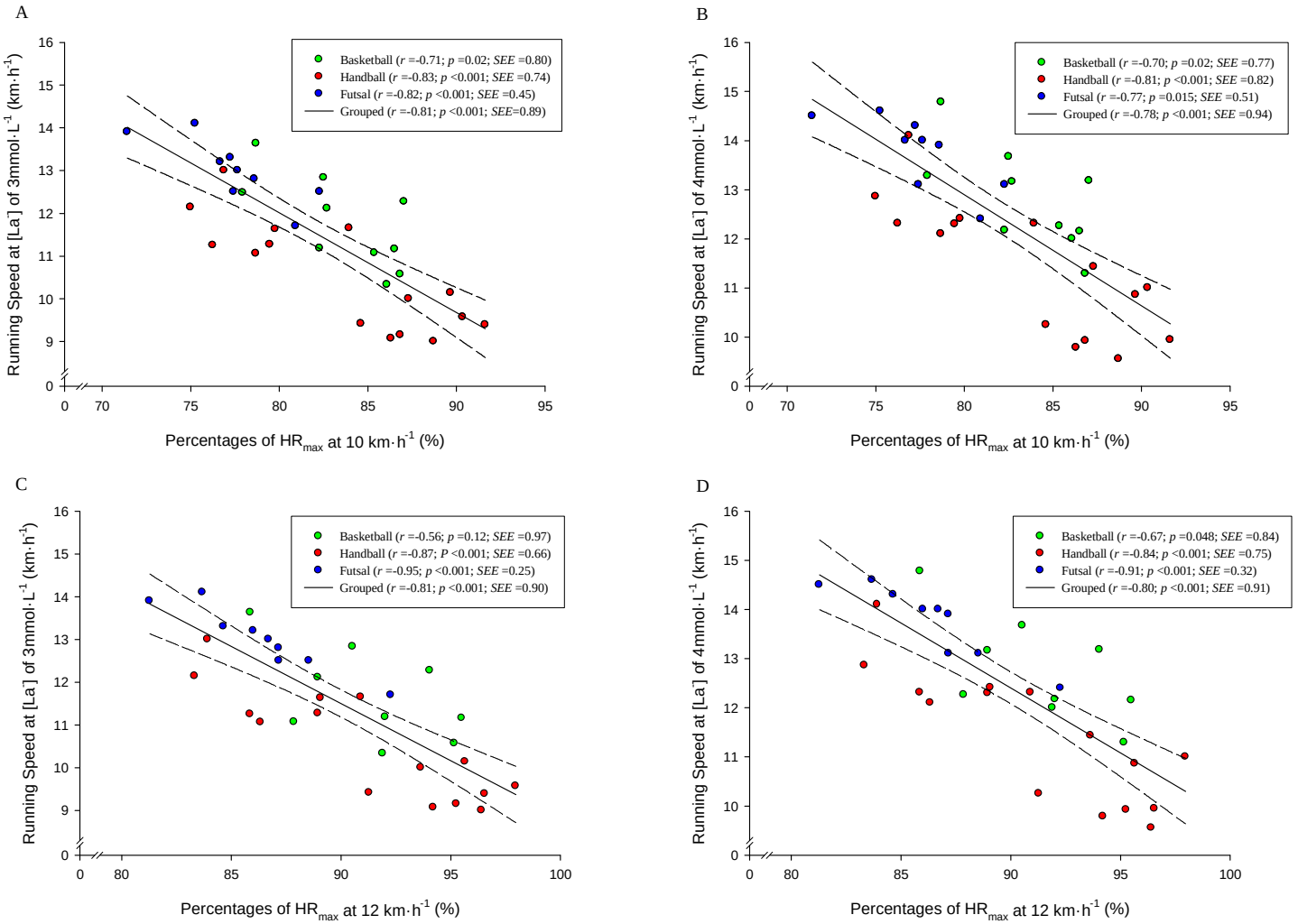

Figure 3: Linear regressions between percentages of maximal heart rate (HR_{max}) at 10 (A, B) and 12 $km \cdot h^{-1}$ (C, D) and the running speeds at blood lactate concentration ($[La^-]$) of 3 $mmol \cdot L^{-1}$ and 4 $mmol \cdot L^{-1}$. Grouped linear regressions solid lines (-); 95% confidence intervals dashed lines (- -)

Discussion

The main finding of this study was that S90%HR_{max} accurately predicted S3mM and S4mM in professional handball, basketball and futsal players. This is the first study reporting the potential of S90%HR_{max} to be used as a simple, low-cost and non-invasive performance variable to frequently assess and monitor aerobic capacity in professional team-sport players.

A careful analysis of the existing literature revealed that S3mM and S4mM, as well as MLSS, usually occur at a mean intensity close to 90% HR_{max} (Mujika, 2012; Kindermann et al., 1979; McMillan et al., 2005; Mujika & Padilla, 2001; Garcia-Tabar et al., 2013; Jones & Doust, 1998). Although it has been suggested that there might be a steady-state relationship between the intensity at a fixed [La^-] and %HR_{max} (Foster et al., 1999; Mujika, 2012), the intensity at a given %HR_{max} as a simple performance variable to predict S3mM and S4mM had never been investigated before. In the present study S90%HR_{max} was close to S3mM and S4mM (Table 2), and it was found to be a good predictor of both fixed lactate thresholds in homogeneous groups of professional team-sport players, particularly in futsal and handball (Figure 2). Likewise, %HR_{max} at 10 and 12 km·h^{-1} were observed to accurately estimate both fixed lactate thresholds (Figure 3). The magnitudes of these correlations are similar to those observed between S4mM and MLSS (Vobejda, Fromme, Samson, & Zimmermann, 2006; Jones & Doust, 1998), and similar or even higher than those observed between individual or fixed lactate thresholds and heart rate deflection points, which, unlike S90%HR_{max}, are not always possible to determine (Bodner & Rhodes, 2000; Vachon, Bassett, Jr., & Clarke, 1999; Hofmann, Bunc, Leitner, Pokan, & Gaisl, 1994). These results indicate that S90%HR_{max} can be used as a non-invasive and easy method to estimate S3mM and S4mM during a progressive running test in professional indoor team-sport players.

The estimations of S3mM and S4mM from S90%HR_{max} were less accurate in basketball than in futsal and handball (Figure 2). Similar results were found when %HR_{max} at 10 and 12 km·h^{-1} were taken as predictor variables (Figure 3). It is well known that the correlation coefficients are influenced by the range in the predictor and responding variables; the greater the range or the heterogeneity of a group the greater the magnitude of the correlation coefficient. In this study, grouped CVs (a normalized measure of dispersion) for S3mM, S4mM and S90%HR_{max} ranged between 10.3 and 12.6% (Table 2). However, when we examined each teams' linear relationships separately, therefore narrowing the range in S3mM, S4mM and S90%HR_{max} (e.g. futsal CVs ranged between 5.2 and 5.5% in S3mM, S4mM and S90%HR_{max}; Table 2), it can be observed that correlation magnitudes augmented in futsal and handball but not in basketball (Figure 2). The non-attainment of a true HR_{max} in some basketball players is suggested to be the main factor explaining these less accurate estimations. Mean differences between HR_{max} and age-predicted HR_{max} (211-0.64·age) were well within ±10.8 b·min^{-1} *SEE* reported for the HR_{max} vs. age relationship (Nes et al., 2013) in every sport team, including basketball. Nevertheless, mean absolute HR_{max} was 8 b·min^{-1} lower ($p < 0.05$) in basketball than in futsal and handball, despite athletes being of similar age. Furthermore, basketball players' mean absolute HR_{max} only corresponded to 95% of their age-predicted HR_{max}, which is lower than the 98% and 99% observed in our futsal and handball players, as well as lower than the 97% to 101% values previously reported in soccer players (Helgerud et al., 2001), long-distance runners (Friedmann et al., 2004), cross-country skiers (Kindermann et al., 1979), amateur or professional cyclists (Mujika & Padilla, 2001) and basketball players (Narazaki, Berg, Stergiou, & Chen, 2009). Lack of motivation and participants'

lack of familiarity with this kind of running tests could have also partly hampered the achievement of a real HR_{max} (Whipp, Davis, Torres, & Wasserman, 1981).While most of the futsal (80%) and handball (77%) players were well accustomed to the exercise protocol, since they were previously tested using the same testing procedures, 70% of the basketball players were not familiar with this test. Other factors such as the effect of the non-standardized warm-up (each team performed their own warm-up routine supervised by their respective physical trainer) or anthropometric, speed and strength characteristics, are thought to be less plausible factors to explain these differences.

The observed relationships between $S90\%HR_{max}$ and both fixed lactate thresholds (Figure 2) and the non-significant differences between $S90\%HR_{max}$ and S3mM and S4mM (Table 2) do not necessarily imply that exercise intensity at either of the fixed lactate thresholds must coincide with the intensity at $90\%HR_{max}$. In fact, the large correlations between S3mM or S4mM and $\%HR_{max}$ at which S3mM or S4mM occurred, suggest that team-sport players with higher fixed lactate thresholds achieve the thresholds at higher $\%HR_{max}$ compared to those with lower fixed lactate thresholds. This is in agreement with previous studies showing that endurance-trained individuals achieve their fixed lactate thresholds at a higher relative load (expressed either as percentage of maximal oxygen uptake or HR_{max}) than less fit individuals (Garcia-Tabar et al., 2013; Hurley et al., 1984). Nonetheless, due to the fact that S3mM and S4mM explained <50% of the variance in $\%HR_{max}$ at their respective fixed lactate thresholds, other factors such as age (Rusko, Rahkila, & Karvinen, 1980) could influence these relationships.

The present study is limited in some aspects. The use of maximal secondary criteria (e.g. perceived exertion or peak $[La^-]$ measures) or a maximal confirmation test could have helped to verify non-attainment of HR_{max}, particularly in basketball. Besides, because steady-state $[La^-]$ can vary among athletes, the assessment of the MLSS may have led to a greater accuracy in the determination of endurance capacities. Nevertheless, the intensity at a given sub-maximal $[La^-]$ accurately predicts endurance capacity (Borch et al., 1993; Heck et al., 1985; Kindermann et al., 1979) and lessens the testing burden on the participant and researcher associated with the MLSS (Mann et al., 2013). The type of exercise protocol used is unlikely to have influenced S3mM or S4mM vs. $\%HR_{max}$ relationships (Whipp et al., 1981), although further corroboration is recommended. Finally, this investigation was conducted on team-sport players with an S4mM between 9.6 $km \cdot h^{-1}$ and 14.8 $km \cdot h^{-1}$ during a specific time of the season that limited the applicability of the results. Due to the fact that individual or fixed lactate thresholds, as well as the MLSS, usually occur at a mean intensity close to 90% HR_{max} regardless of the level of aerobic capacity of the individuals (Garcia-Tabar et al., 2013; Jones & Doust, 1998; Kindermann et al., 1979; McMillan et al., 2005; Mujika & Padilla, 2001; Mujika, 2012), and that this relationship is maintained despite alterations in the intensity of the individual or fixed lactate thresholds due to training, detraining or hypoxia (Foster et al., 1999; Friedmann et al., 2004; Helgerud et al., 2001; Hurley et al., 1984; Lucia et al., 2000; Mujika, 2012; McMillan et al., 2005), $S90\%HR_{max}$ might be a useful variable to predict and monitor fixed lactate thresholds during an entire competitive season and in other populations with an S4mM lower than 9.6 $km \cdot h^{-1}$ or higher than 14.8 $km \cdot h^{-1}$. However, whether a steady-state relationship between fixed lactate thresholds and $\%HR_{max}$ is maintained throughout an entire competitive season, in athletes of other sports, in athletes with different level of aerobic capacity or under other conditions (e.g. glycogen depleted state or hypoxia) remains unclear, is beyond the scope of this study, and deserves further research.

Practical Applications

This study supports that aerobic capacity can be assessed and monitored through S90%HR_{max} in basketball, handball and futsal players with an S4mM between 9.6 km·h^{-1} and 14.8 km·h^{-1}. The use of S90%HR_{max} as an endurance performance variable could facilitate the assessment and monitoring of aerobic capacity in team-sports and coaches with limited resources. Indeed, this variable is a simple, low-cost and non-invasive variable that allows investigation of several players at the same time without the need of expensive equipment or technical expertise to administer the test. Further research to confirm these results in other sports composed of athletes with an S4mM lower than 9.6 km·h^{-1} or higher than 14.8 km·h^{-1} and to explore the possible physiological mechanisms underpinning the fixed lactate thresholds and S90%HR_{max} relationships is warranted.

Acknowledgments

None of the authors declare any relationships with companies or manufactures that would benefit from the results of this study. The results of the present study do not constitute endorsement of the product by the authors or the NSCA.

References

Alvarez, J. C., D'Ottavio, S., Vera, J. G., & Castagna, C. (2009). Aerobic fitness in futsal players of different competitive level. *J Strength.Cond.Res., 23,* 2163-2166.

Ben Abdelkrim, N., El Fazaa, S., & El Ati, J. (2007). Time-motion analysis and physiological data of elite under-19-year-old basketball players during competition. *Br.J Sports Med., 41,* 69-75.

Beneke, R. (1995). Anaerobic threshold, individual anaerobic threshold, and maximal lactate steady state in rowing. *Med.Sci.Sports Exerc., 27,* 863-867.

Bodner, M. E. & Rhodes, E. C. (2000). A review of the concept of the heart rate deflection point. *Sports Med., 30,* 31-46.

Borch, K. W., Ingjer, F., Larsen, S., & Tomten, S. E. (1993). Rate of accumulation of blood lactate during graded exercise as a predictor of 'anaerobic threshold'. *J Sports Sci., 11,* 49-55.

Foster, C., Fitzgerald, D. J., & Spatz, P. (1999). Stability of the blood lactate-heart rate relationship in competitive athletes. *Med.Sci.Sports Exerc, 31,* 578-582.

Friedmann, B., Bauer, T., Menold, E., & Bartsch, P. (2004). Exercise with the intensity of the individual anaerobic threshold in acute hypoxia. *Med.Sci.Sports Exerc, 36,* 1737-1742.

Garcia-Tabar, I., Sánchez-Medina, L., Aramendi, J., Ruesta, M., Ibañez, J., & Gorostiaga, E. (2013). Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists. *J Exerc Physiol Online, 16,* 38-50.

Gorostiaga, E. M., Granados, C., Ibanez, J., Gonzalez-Badillo, J. J., & Izquierdo, M. (2006). Effects of an entire season on physical fitness changes in elite male handball players. *Med.Sci.Sports Exerc, 38,* 357-366.

Gorostiaga, E. M., Granados, C., Ibanez, J., & Izquierdo, M. (2005). Differences in physical fitness and throwing velocity among elite and amateur male handball players. *Int.J Sports Med., 26,* 225-232.

Gorostiaga, E. M., Llodio, I., Ibanez, J., Granados, C., Navarro, I., Ruesta, M. et al. (2009). Differences in physical fitness among indoor and outdoor elite male soccer players. *Eur J Appl Physiol, 106,* 483-491.

Granados, C., Izquierdo, M., Ibanez, J., Ruesta, M., & Gorostiaga, E. M. (2008). Effects of an entire season on physical fitness in elite female handball players. *Med.Sci.Sports Exerc, 40,* 351-361.

Hauser, T., Adam, J., & Schulz, H. (2014). Comparison of Selected Lactate Threshold Parameters with Maximal Lactate Steady State in Cycling. *Int.J Sports Med., 35,* 517-521.

Heck, H., Mader, A., Hess, G., Mucke, S., Muller, R., & Hollmann, W. (1985). Justification of the 4-mmol/l lactate threshold. *Int.J Sports Med., 6,* 117-130.

Helgerud, J., Engen, L. C., Wisloff, U., & Hoff, J. (2001). Aerobic endurance training improves soccer performance. *Med.Sci.Sports Exerc, 33,* 1925-1931.

Hofmann, P., Bunc, V., Leitner, H., Pokan, R., & Gaisl, G. (1994). Heart rate threshold related to lactate turn point and steady-state exercise on a cycle ergometer. *Eur J Appl Physiol Occup.Physiol, 69,* 132-139.

Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Med.Sci.Sports Exerc, 41,* 3-13.

Hurley, B. F., Hagberg, J. M., Allen, W. K., Seals, D. R., Young, J. C., Cuddihee, R. W. et al. (1984). Effect of training on blood lactate levels during submaximal exercise. *J Appl Physiol Respir.Environ.Exerc Physiol, 56,* 1260-1264.

Jones, A. M. & Doust, J. H. (1998). The validity of the lactate minimum test for determination of the maximal lactate steady state. *Med.Sci.Sports Exerc, 30,* 1304-1313.

Kindermann, W., Simon, G., & Keul, J. (1979). The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur J Appl Physiol Occup.Physiol, 42,* 25-34.

Kuipers, H., Rietjens, G., Verstappen, F., Schoenmakers, H., & Hofman, G. (2003). Effects of stage duration in incremental running tests on physiological variables. *Int.J.Sports Med., 24,* 486-491.

Loures, J. P., Chamari, K., Ferreira, E. C., Campos, E. Z., Zagatto, A. M., Milioni, F. et al. (2015). Specific determination of maximal lactate steady state in soccer players. *J.Strength.Cond.Res., 29,* 101-106.

Lucia, A., Hoyos, J., Perez, M., & Chicharro, J. L. (2000). Heart rate and performance parameters in elite cyclists: a longitudinal study. *Med.Sci.Sports Exerc., 32,* 1777-1782.

Mann, T., Lamberts, R. P., & Lambert, M. I. (2013). Methods of prescribing relative exercise intensity: physiological and practical considerations. *Sports Med., 43,* 613-625.

McMillan, K., Helgerud, J., Grant, S. J., Newell, J., Wilson, J., Macdonald, R. et al. (2005). Lactate threshold responses to a season of professional British youth soccer. *Br.J Sports Med., 39,* 432-436.

Mujika, I. (2012). The cycling physiology of Miguel Indurain 14 years after retirement. *Int.J Sports Physiol Perform., 7,* 397-400.

Mujika, I. & Padilla, S. (2001). Physiological and performance characteristics of male professional road cyclists. *Sports Med., 31,* 479-487.

Narazaki, K., Berg, K., Stergiou, N., & Chen, B. (2009). Physiological demands of competitive basketball. *Scand.J Med.Sci.Sports, 19,* 425-432.

Nes, B. M., Janszky, I., Wisloff, U., Stoylen, A., & Karlsen, T. (2013). Age-predicted maximal heart rate in healthy subjects: The HUNT Fitness Study. *Scand.J.Med.Sci.Sports, 23,* 697-704.

Pfitzinger, P. & Freedson, P. S. (1998). The reliability of lactate measurements during exercise. *Int.J Sports Med., 19,* 349-357.

Povoas, S. C., Seabra, A. F., Ascensao, A. A., Magalhaes, J., Soares, J. M., & Rebelo, A. N. (2012). Physical and physiological demands of elite team handball. *J Strength.Cond.Res., 26,* 3365-3375.

Rusko, H., Luhtanen, P., Rahkila, P., Viitasalo, J., Rehunen, S., & Harkonen, M. (1986). Muscle metabolism, blood lactate and oxygen uptake in steady state exercise at aerobic and anaerobic thresholds. *Eur.J.Appl.Physiol Occup.Physiol, 55,* 181-186.

Rusko, H., Rahkila, P., & Karvinen, E. (1980). Anaerobic threshold, skeletal muscle enzymes and fiber composition in young female cross-country skiers. *Acta Physiol Scand., 108,* 263-268.

Sjödin, B. & Jacobs, I. (1981). Onset of blood lactate accumulation and marathon running performance. *Int.J Sports Med., 2,* 23-26.

Vachon, J. A., Bassett, D. R., Jr., & Clarke, S. (1999). Validity of the heart rate deflection point as a predictor of lactate threshold during running. *J Appl Physiol (1985.), 87,* 452-459.

Vobejda, C., Fromme, K., Samson, W., & Zimmermann, E. (2006). Maximal constant heart rate--a heart rate based method to estimate maximal lactate steady state in running. *Int.J Sports Med., 27,* 368-372.

Weltman, A., Snead, D., Stein, P., Seip, R., Schurrer, R., Rutt, R. et al. (1990). Reliability and validity of a continuous incremental treadmill protocol for the determination of lactate threshold, fixed blood lactate concentrations, and VO2max. *Int.J Sports Med., 11,* 26-32.

Whipp, B. J., Davis, J. A., Torres, F., & Wasserman, K. (1981). A test to determine parameters of aerobic function during exercise. *J Appl Physiol Respir.Environ.Exerc Physiol, 50,* 217-221.

Ziv, G. & Lidor, R. (2009). Physical attributes, physiological characteristics, on-court performances and nutritional strategies of female and male basketball players. *Sports Med., 39,* 547-568.

Chapter 4

Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists

* Garcia-Tabar I, Sánchez-Medina L, Aramendi JF, Ruesta M, Ibañez J & Gorostiaga EM. Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists. *Journal of Exercise Physiology Online* 2013;16(5):38-50

Heart rate variability thresholds predict lactate thresholds in professional world-class road cyclists

Authors

Ibai Garcia-Tabar, Luis Sánchez-Medina, José F. Aramendi, Maite Ruesta, Javier Ibañez & Esteban M. Gorostiaga

Affiliation

Studies, Research and Sports Medicine Center, Government of Navarre, Pamplona, Spain

Abstract

This study aimed to predict widely used aerobic (AeT) and anaerobic lactate thresholds (AnTs) and other cycling performance variables from mathematically determined heart rate variability thresholds (HRVTs). Twelve male professional world-class road cyclists performed a continuous maximal graded cycling test. Blood lactate concentration, heart rate (HR) and RR intervals were monitored. Four different LTs (one AeT and three AnTs) were determined. HRVTs were determined from the standard deviation of the instantaneous beat-to-beat RR intervals (SD1). The AeT and one of the HRVT were not statistically different. Significant relationships (P <0.05) were found between the lactate thresholds and the HRVTs (r = 0.65-0.88). HRVTs strongly correlated with percentages of peak aerobic power (r = 0.94-0.97; P <0.001) and percentages of peak HR (r = 0.87-0.95; P <0.001) at which these thresholds occurred. Results indicated that lactate thresholds and percentages of peak aerobic power and peak HR at the HRVTs can be accurately predicted from SD1 values during a maximal or submaximal, non-invasive, low-cost, incremental exercise test in world-class road cyclists. The AeT might be coincidental with the vagal withdrawal of the heart.

Keywords:Heart Rate Monitor, Cardiac Vagal Activity, Training, Elite Athletes, Exercise Testing

Chapter 5

Validity of a single lactate measure to predict fixed lactate thresholds in elite athletes

* Garcia-Tabar I, Llodio I, Sánchez-Medina L, Asiain X., Ibañez J & Gorostiaga EM. Validity of a single lactate measure to predict fixed lactate thresholds in elite athletes. *Journal of Sports Sciences* 2016; 35(4): 385-392. DOI: 10.1080/02640414.2016.1166392

Validity of a single lactate measure to predict fixed lactate thresholds in athletes

Authors

Ibai Garcia-Tabar[1], Iñaki Llodio[1], Luis Sánchez-Medina[1], Xabier Asiain[2], Javier Ibáñez[1], Esteban M. Gorostiaga[1]

Affiliation

[1]Studies, Research and Sports Medicine Centre, Government of Navarre, Pamplona, Spain

[2]Spanish Basque-ball Federation, Madrid, Spain

Abstract

This study aimed to validate the use of a single blood lactate concentration measure taken following a 12 $km \cdot h^{-1}$ running stage (BLC_{12}) to predict and monitor fixed blood lactate concentration (FBLC) thresholds. Three complementary studies were undertaken. *Study I*: the relationships between BLC_{12} and the running speeds at FBLC of 3 $mmol \cdot L^{-1}$ (S3mM) and 4 $mmol \cdot L^{-1}$ (S4mM) measured during a multistage running field test were examined in 136 elite athletes. *Study II*: data from 30 athletes tested one year apart were used to test the predictive capacity of the equations obtained in *Study I. Study III*: 80 athletes were tested before and after an intensified training period to examine whether training-induced changes in FBLC thresholds could be predicted and monitored by BLC_{12}. *Study I*: BLC_{12} was significantly ($P<0.001$) and inversely related to S3mM ($R^2=0.89$) and S4mM ($R^2=0.95$). *Study II:* prediction models yielded robust correlations between the estimated and measured FBLC thresholds ($r=0.94$ to 0.99; $P<0.001$). *Study III*: estimated changes predicted actual training-induced changes in FBLC thresholds ($r=0.81$ to 0.91; $P<0.001$). This study gives empirical support to use a single lactate measure during a sub-maximal running field test as a simple, low-cost and practical alternative to FBLC thresholds in athletes.

Keywords: OBLA, anaerobic threshold, endurance training, exercise testing, elite athletes

Chapter 6

Main results, conclusions, practical applications and future perspectives

Brief summary remainder

Among the international exercise and sports physiology community, main determinants of endurance exercise performance, and therefore, main determinants of aerobic capacity, are considered to be 1) maximal oxygen uptake ($\dot{V}o_{2max}$), 2) the so called "*lactate threshold*" (LT) or maximal lactate steady state (MLSS), and 3) work efficiency (Beneke, 1995; Coyle, 1995; Joyner & Coyle, 2008). In the context of sports physiology, aerobic capacity is mainly determined for scientific support given to endurance athletes or physical activity practitioners. Thus, the development of practical strategies to overcome limitations encountered during regular longitudinal aerobic capacity monitoring is of great interest for sport practitioners. Notwithstanding, aerobic capacity is also widely determined during research studies. In our particular case, LT or MLSS estimations are routinely measured during laboratory- and field-based regular endurance assessments, while $\dot{V}o_{2max}$ is measured primarily during laboratory-based research projects. The current Ph.D. dissertation, hence, focused on the determination of aerobic capacity by means of $\dot{V}o_{2max}$ and LT testing, with special reference to the development of practical strategies to overcome on-field actual hitches that we (sport scientific practitioners) might confront at the time to give scientific endurance support to athletes or physical activity practitioners.

Aerobic capacity has traditionally been determined by means of $\dot{V}o_{2max}$ testing, since $\dot{V}o_{2max}$ was the first (Bassett & Howley, 1997; Hawkins, Raven, Snell, Stray-Gundersen, & Levine, 2007; Levine, 2008) physiological variable found to be well correlated to endurance performance in different sport disciplines (Astrand & Saltin, 1961a; Astrand & Saltin, 1961b; Saltin & Astrand, 1967). Thanks to the development of automated metabolic systems (Beaver, Wasserman, & Whipp, 1973; Kissen & McGuire, 1967), $\dot{V}o_{2max}$ testing is more reasoable and practical today, and it is commonly use to assess endurance performance in athletes (Capostagno, Lambert, & Lamberts, 2016), to prescribe exercise training (Mann, Lamberts, & Lambert, 2013), to guide treatment in clinical patients (Nelson & Asplund, 2016) and to predict prognosis and mortality (Myers et al., 2002). However, there are many potential errors related to the technology of these automated systems that are difficult to control and detect (Gore, Tanner, Fuller, & Stanef, 2013; Howley, Bassett, & Welch, 1995). One of the main potential sources of error in the calculation of $\dot{V}o_{2max}$ using automated systems is related to the stability of fractional concentrations of oxygen (FO_2) and carbon dioxide (FCO_2) measurements, because the electronic oxygen (O_2) and carbon dioxide (CO_2) analyzers are prone to drift over time. It is generally assume that respiratory measures are valid as long as pre-test calibration procedures are followed, and clearly, the process of after trial verification tends to be overlooked.

In our **Study 1** (Chapter 2) presented in this thesis we examined the drift in the measurements of FO_2 and FCO_2 during incremental exercise to exhaustion, and determined the influence of the drift on the determination of $\dot{V}o_{2max}$. We also proposed a simple way to correct the error in $\dot{V}o_{2max}$ determination associated with the drift.

As abovementioned, during the 1950s and 1960s, primarily by the studies reported by Astrand and Saltin [eg. Astrand and Saltin (1961a); Saltin and Astrand (1967)], $\dot{V}o_{2max}$ was consolidated as a determinant factor of endurance performance. Nevertheless, the value of $\dot{V}o_{2max}$ as a predictor of endurance performance in homogenous samples of athletes soon came up to debate

(Conley & Krahenbuhl, 1980; Farrell, Wilmore, Coyle, Billing, & Costill, 1979). Since then, $\dot{V}o_{2max}$ has been robustly confirmed to be rather an insensitive variable to detect endurance performance improvements in elite endurance athletes [eg. Coyle (2005); Jones (1998)]. The development of enzymatic micro-methods for measuring blood lactate concentration (BLC) from capillary blood samples, together with the large amount of studies demonstrating robust correlations between lactate thresholds (LTs) and endurance performance (Faude, Kindermann & Meyer, 2009), turned LT testing more popular than respiratory gases for the assessment of endurance performance in athletes (Roecker, Schotte, Niess, Horstmann, & Dickhuth, 1998). According to Faude, Kindermann and Meyer (2009), nowadays there are more than 25 LT concepts. LT determination, thereby, requires qualified personnel not only for invasive blood sampling, but also for correct interpretation and appropriate handling of the results. The need of qualified professionals often hinders frequent (weekly or monthly) LT determination as would be required for ongoing monitoring of the aerobic capacity and proper adjustments in endurance training intensity, particularly in individuals with limited resources. It seems therefore worthwhile to scientifically evaluate methods for LTs' determination requiring minimal equipment.

Study 2 (Chapter 3), **Study 3** (Chapter 4) and **Study 4** (Chapter 5) presented in this doctoral thesis are field-based studies carried out during regular aerobic capacity monitoring of elite and professional (some world-class) athletes having financial support for LT testing. The primary aim of these three studies was to develop functional (low-cost, simple and time-efficient) alternatives to LTs determination in order to facilitate the determination and monitoring of aerobic capacity.

Main results, conclusions, practical applications and future perspectives

Study 1 (Chapter 2)

The aim of the first study was to examine the drift in the measurements of FO_2 and FCO_2 of a Nafion-using metabolic cart during incremental maximal exercise in 18 young and 12 elderly males.

> **Result 1:** Under controlled laboratory conditions, we found a physiologically significant downscale drift in FO_2 and FCO_2 at the end of maximal exercise. The drift was not due to an electronic instability in the analyzers because it was reverted after 20 minutes of recovery from the end of the exercise. The most likely explanation for the drift is an accumulation of excess water vapor in the sample line which could not be completely removed during transit through the Nafion conducting tube. The correction method proposed indicates that ignoring the effects of the drift would induce an average $\dot{V}o_{2max}$ overestimation of 5-6% and a maximal respiratory exchange ratio (RER_{max}) underestimation of 8-9%, with errors ranging up to 11-12% and 15-16% in $\dot{V}o_{2max}$ and RER_{max}, respectively.
>
> **Conclusion 1:** The disagreement between the measured and the corrected $\dot{V}o_{2max}$ and RER_{max} values observed in our particular metabolic system is not acceptable to test athletes, to prescribe exercise intensities, to calculate the fat oxidation rate from RER values, or to use the respiratory values for some other clinical purposes, such as to guide treatment in patients with chronic heart failure, to enter in cardiac transplantation listing, to indicate the health status or to predict prognosis and mortality.

Practical application 1: The implications of the present study point to the necessity to check FO_2 and FCO_2 values by carefully calibrating the pre-test calibration gases and verifying a possible shift immediately after exercise, as well as to correct the respiratory data in situations where the drift in O_2 and CO_2 analyzers occurs. Special care must be taken in studies where a Nafion conducting tube is used.

Future perspective 1: Further research in this area is certainly warranted to establish valid correction factors for each device. A wider study is needed to extend the present findings to the wide metabolic systems' population.

Study 2 (Chapter 3)

The aim of the second study was to investigate whether the speed associated with 90% of maximal heart rate ($S90\%HR_{max}$) could predict the speeds associated with the widely used fixed blood lactate concentration (FBLC) thresholds of 3 $mmol \cdot L^{-1}$ (S3mM) and 4 $mmol \cdot L^{-1}$ (S4mM).

Result 2: $S90\%HR_{max}$ was found to be robustly correlated with both FBLC thresholds in 36 professional team-sport players during an incremental discontinuous exercise test.

Conclusion 2: Aerobic capacity can be assessed and monitored through $S90\%HR_{max}$ in basketball, handball and futsal players with an S4mM between 9.6 $km \cdot h^{-1}$ and 14.8 $km \cdot h^{-1}$.

Practical application 2: The use of $S90\%HR_{max}$ as an endurance performance variable could facilitate the assessment and monitoring of aerobic capacity in team-sports and coaches with limited resources. Indeed, $S90\%HR_{max}$ is a simple, low-cost and non-invasive variable that allows investigation of several players at the same time without the need of expensive equipment or technical expertise to administer the test.

Future perspective 2: Further research to confirm these results in other sports composed of athletes with an S4mM lower than 9.6 $km \cdot h^{-1}$ or higher than 14.8 $km \cdot h^{-1}$ and to explore the possible physiological mechanisms underpinning the FBLC thresholds and $S90\%HR_{max}$ relationships is warranted. Whether a steady-state relationship between FBLC thresholds and $S90\%HR_{max}$ is maintained throughout an entire competitive season, in athletes of other sports, in athletes with different level of aerobic capacity or under other conditions (e.g. glycogen depleted state or hypoxia) remains unclear, was beyond the scope of this study, and deserves further research.

Study 3 (Chapter 4)

In the third study we proposed three new mathematical determined heart rate variability (HRV) thresholds to estimate the LTs in 12 professional world-class road cyclists.

Result 3: Large to very large relationships were found between the LTs and the HRV thresholds during the continuous maximal graded cycling test performed by 12 professional world-class cyclists.

Conclusion 3: LTs can be precisely and objectively estimated from HRV measures during an incremental exercise test in a homogeneous group of professional male world-class road cyclists. The aerobic LT might coincide with the vagal withdrawal of the heart.

Practical application 3: Prediction of LTs by HRV through a non-invasive, maximal or submaximal, low-cost, incremental exercise test measurable in non-laboratory settings without the need of any technical expertise to administer the test could facilitate aerobic capacity monitoring in professional cyclists.

Future perspective 3: This investigation was conducted on a homogenous group of athletes during a specific time of the season that limited the applicability of the results. Further research is warranted to confirm the associations between the LT and HRV in other periods of the season and in other populations.

Study 4 (Chapter 5)

The last study validated the use of a single blood lactate concentration (BLC) measure taken following a 12 $km \cdot h^{-1}$ running stage to predict and monitor FBLC thresholds in 136 elite male athletes.

Result 4: BLC at a running speed of 12 $km \cdot h^{-1}$ (BLC_{12}) was demonstrated to be a strong predictor of the running speeds associated with FBLC of 3 $mmol \cdot L^{-1}$ (S3mM) and 4 $mmol \cdot L^{-1}$ (S4mM) in 136 elite athletes. The accuracy of the obtained prediction equations was preserved in 30 athletes that were re-tested in the following year. The BLC_{12}-S3mM and BLC_{12}-S4mM relationships were maintained after an intensified training period, despite improvements in S3mM and S4mM, and were found to be very useful to track training-induced changes in both FBLC thresholds.

Conclusion 4: Results found support he use a single lactate measure during a two-stage 13-min sub-maximal running field test as a simple, low-cost and practical alternative to FBLC thresholds in athletes.

Practical application 4: The quick execution of the proposed testing protocol (13 min), its low-cost, its sub-maximal nature and the high accuracy of the prediction equations to estimate S3mM and S4mM could facilitate the assessment and monitoring of aerobic capacity in athletes from different sports. The reported prediction equations are only recommended to be used with the specific testing procedures utilized and described in this study.

Future perspective 4: Whether the relationships between BLC_{12} and S3mM or S4mM are maintained in athletes with S4mM values lower than 9.2 $km \cdot h^{-1}$ or higher than 15.1 $km \cdot h^{-1}$ is a question that remains unknown and was beyond the scope of this study. The same holds true for gender, because specific prediction models have not been developed for females. Further validation of the prediction equations presented in this study would be needed in different athletic populations and in different gender and age specific samples before this test can be established for mass field testing.

References

Astrand, P. O. & Saltin, B. (1961a). Maximal oxygen uptake and heart rate in various types of muscular activity. *J Appl.Physiol, 16,* 977-981.

Astrand, P. O. & Saltin, B. (1961b). Oxygen uptake during the first minutes of heavy muscular exercise. *J Appl.Physiol, 16,* 971-976.

Bassett, D. R. Jr. & Howley, E. T. (1997). Maximal oxygen uptake: "classical" versus "contemporary" viewpoints. *Med.Sci.Sports Exerc, 29,* 591-603.

Beaver, W. L., Wasserman, K., & Whipp, B. J. (1973). On-line computer analysis and breath-by-breath graphical display of exercise function tests. *J Appl.Physiol, 34,* 128-132.

Beneke, R. (1995). Anaerobic threshold, individual anaerobic threshold, and maximal lactate steady state in rowing. *Med Sci.Sports Exerc, 27,* 863-867.

Capostagno, B., Lambert, M. I., & Lamberts, R. P. (2016). Systematic Review of Submaximal Cycle Tests to Predict, Monitor and Optimize Cycling Performance. *Int.J Sports Physiol Perform.*.

Conley, D. L. & Krahenbuhl, G. S. (1980). Running economy and distance running performance of highly trained athletes. *Med Sci.Sports Exerc, 12,* 357-360.

Coyle, E. F. (1995). Integration of the physiological factors determining endurance performance ability. *Exerc Sport Sci.Rev., 23,* 25-63.

Coyle, E. F. (2005). Improved muscular efficiency displayed as Tour de France champion matures. *J Appl.Physiol (1985.), 98,* 2191-2196.

Farrell, P. A., Wilmore, J. H., Coyle, E. F., Billing, J. E., & Costill, D. L. (1979). Plasma lactate accumulation and distance running performance. *Med Sci.Sports, 11,* 338-344.

Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate threshold concepts: how valid are they? *Sports Med, 39,* 469-490.

Gore, C. J., Tanner, R. K., Fuller, K. L., & Stanef, F. (2013). Determination of maximal oxygen consumption (VO2max). In R.K.Tanner & C. J. Gore (Eds.), *Physiological tests for elite athletes* (pp. 103-122). Champaign, IL: Human Kinetics.

Hawkins, M. N., Raven, P. B., Snell, P. G., Stray-Gundersen, J., & Levine, B. D. (2007). Maximal oxygen uptake as a parametric measure of cardiorespiratory capacity. *Med.Sci.Sports Exerc, 39,* 103-107.

Howley, E. T., Bassett, D. R. Jr., & Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Med.Sci.Sports Exerc., 27,* 1292-1301.

Jones, A. M. (1998). A five year physiological case study of an Olympic runner. *Br.J Sports Med, 32,* 39-43.

Joyner, M. J. & Coyle, E. F. (2008). Endurance exercise performance: the physiology of champions. *J Physiol, 586,* 35-44.

Kissen, A. T. & McGuire, D. W. (1967). New approach for on-line, continuous determination of oxygen consumption in human subjects. *Aerosp.Med, 38,* 686-689.

Levine, B. D. (2008). .VO2max: what do we know, and what do we still need to know? *J Physiol, 586,* 25-34.

Mann, T., Lamberts, R. P., & Lambert, M. I. (2013). Methods of prescribing relative exercise intensity: physiological and practical considerations. *Sports Med, 43,* 613-625.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, J. E. (2002). Exercise capacity and mortality among men referred for exercise testing. *N.Engl.J Med, 346,* 793-801.

Nelson, N. & Asplund, C. A. (2016). Exercise Testing: Who, When, and Why? *PM.R., 8,* S16-S23.

Roecker, K., Schotte, O., Niess, A. M., Horstmann, T., & Dickhuth, H. H. (1998). Predicting competition performance in long-distance running by means of a treadmill test. *Med Sci.Sports Exerc, 30,* 1552-1557.

Saltin, B. & Astrand, P. O. (1967). Maximal oxygen uptake in athletes. *J Appl.Physiol, 23,* 353-358.

Ibai